



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C07K 16/00</p>	A2	<p>(11) International Publication Number: WO 00/37504</p> <p>(43) International Publication Date: 29 June 2000 (29.06.00)</p>												
<p>(21) International Application Number: PCT/US99/30895</p> <p>(22) International Filing Date: 23 December 1999 (23.12.99)</p> <p>(30) Priority Data: 60/113,647 23 December 1998 (23.12.98) US</p> <p>(71) Applicants (for all designated States except US): PFIZER, INC. [US/US]; Easternpoint Road, Groton, CT 06340 (US). AB-GENIX, INC. [US/US]; 7601 Dumbarton Circle, Fremont, CA 94555 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): HANSON, Douglas, Charles [US/US]; 3 Acom Drive, Niantic, CT 06357 (US). NEVEU, Mark, Joseph [US/US]; 18 Greenbriar Court, Mystic, CT 06355 (US). MUELLER, Eileen, Elliott [US/US]; 4 Butterwick Lane, Old Lyme, CT 06371 (US). HANKE, Jeffrey, Herbert [US/US]; 1 Jefferson Circle, Reading, MA 01867 (US). GILMAN, Steven, Christopher [US/US]; 118 Sill Lane, Old Lyme, CT 06371 (US). DAVIS, C., Geoffrey [US/US]; 1132 Vancouver Avenue, Burlingame, CA 94010 (US). CORVALAN, Jose, Ramon [CL/US]; 125 Williams Lane, Foster City, CA 94010 (US).</p>														
<p>(74) Agent: HARE, Christopher, A.; Abgenix, Inc., 7601 Dumbarton Circle, Fremont, CA 94555 (US).</p> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>														
<p>(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CTLA-4</p>														
<p style="text-align: center;">Enhancement of IL-2 Production Induced by Anti-CTLA4 MABs (30 µg/ml) in the 72 Hour T Blast / Raji and Superantigen Assays (6 Donors)</p> <table border="1" style="margin: 10px auto; border-collapse: collapse; text-align: center;"> <caption>Approximate IL-2 Enhancement (pg/ml) ± SEM</caption> <thead> <tr> <th>Assay</th> <th>CT4.1.1 (pg/ml)</th> <th>CT11.2.1.4 (pg/ml)</th> </tr> </thead> <tbody> <tr> <td>T Blast / Raji</td> <td>~4800</td> <td>~4500</td> </tr> <tr> <td>SEA / PBMC</td> <td>~2500</td> <td>~2000</td> </tr> <tr> <td>SEA / Blood</td> <td>~9000</td> <td>~8500</td> </tr> </tbody> </table>			Assay	CT4.1.1 (pg/ml)	CT11.2.1.4 (pg/ml)	T Blast / Raji	~4800	~4500	SEA / PBMC	~2500	~2000	SEA / Blood	~9000	~8500
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HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

BACKGROUND OF THE INVENTION

5 1. Cross-Reference to Related Applications

The present application claims priority to U.S. Provisional Patent Application, Serial No. 60/113,647, filed December 23, 1998, the disclosure of which is hereby incorporated in its entirety herein.

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2. Summary of the Invention

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4
15 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are
20 antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.

3. Background of the Technology

25 Regulation of immune response in patients would provide a desirable treatment of many human diseases that could lead to a specificity of action that is rarely found through the use of conventional drugs. Both up-regulation and down-regulation of responses of the immune system would be possible. The roles of T cells and B cells have been extensively studied and characterized in
30 connection with the regulation of immune response. From these studies, the role of T cells appear, in many cases, to be particularly important in disease prevention and treatment.

T cells possess very complex systems for controlling their interactions. Interactions between T cells utilize numerous receptors and soluble factors for the process. Thus, what effect any particular signal may have on the immune response generally varies and depends on the particular factors, receptors and counter-receptors that are involved in the pathway. The pathways for down-regulating responses are as important as those required for activation. Thymic education leading to T-cell tolerance is one mechanism for preventing an immune response to a particular antigen. Other mechanisms, such as secretion of suppressive cytokines, are also known.

Activation of T cells requires not only stimulation through the antigen receptor (T cell receptor (TCR)), but additional signaling through co-stimulatory surface molecules such as CD28. The ligands for CD28 are the B7-1 (CD80) and B7-2 (CD86) proteins, which are expressed on antigen-presenting cells such as dendritic cells, activated B-cells or monocytes that interact with T-cell CD28 or CTLA-4 to deliver a costimulatory signal. The role of costimulatory signaling was studied in experimental allergic encephalomyelitis (EAE) by Perrin et al. *Immunol Res* 14:189-99 (1995). EAE is an autoimmune disorder, induced by Th1 cells directed against myelin antigens that provides an *in vivo* model for studying the role of B7-mediated costimulation in the induction of a pathological immune response. Using a soluble fusion protein ligand for the B7 receptors, as well as monoclonal antibodies specific for either CD80 or CD86, Perrin et al. demonstrated that B7 costimulation plays a prominent role in determining clinical disease outcome in EAE.

The interaction between B7 and CD28 is one of several co-stimulatory signaling pathways that appear to be sufficient to trigger the maturation and proliferation of antigen specific T-cells. Lack of co-stimulation, and the concomitant inadequacy of IL-2 production, prevent subsequent proliferation of the T cell and induce a state of non-reactivity termed "anergy". A variety of viruses and tumors may block T cell activation and proliferation, leading to insufficient activity or non-reactivity of the host's immune system to the infected or transformed cells. Among a number of possible T-cell disturbances,

anergy may be at least partly responsible for the failure of the host to clear the pathogenic or tumorigenic cells.

The use of the B7 protein to mediate anti-tumor immunity has been described in Chen et al. *Cell* 71:1093-1102 (1992) and Townsend and Allison
5 *Science* 259:368 (1993). Schwartz *Cell* 71:1065 (1992) reviews the role of CD28, CTLA-4, and B7 in IL-2 production and immunotherapy. Harding et al. *Nature* 356:607-609 (1994) demonstrates that CD28 mediated signaling co-stimulates murine T cells and prevents the induction of anergy in T cell clones. See also U.S. Patent Nos. 5,434,131, 5,770,197, and 5,773,253, and
10 International Patent Application Nos. WO 93/00431, WO 95/01994, WO 95/03408, WO 95/24217, and WO 95/33770.

From the foregoing, it was clear that T-cells required two types of signals from the antigen presenting cell (APC) for activation and subsequent differentiation to effector function. First, there is an antigen specific signal
15 generated by interactions between the TCR on the T-cell and MHC molecules presenting peptides on the APC. Second, there is an antigen-independent signal that is mediated by the interaction of CD28 with members of the B7 family (B7-1 (CD80) or B7-2 (CD86)). Exactly where CTLA-4 fit into the milieu of immune responsiveness was initially evasive. Murine CTLA-4 was first
20 identified and cloned by Brunet et al. *Nature* 328:267-270 (1987), as part of a quest for molecules that are preferentially expressed on cytotoxic T lymphocytes. Human CTLA-4 was identified and cloned shortly thereafter by Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988). The murine and human CTLA-4 molecules possess approximately 76% overall sequence homology and
25 approach complete sequence identity in their cytoplasmic domains (Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988)). CTLA-4 is a member of the immunoglobulin (Ig) superfamily of proteins. The Ig superfamily is a group of proteins that share key structural features of either a variable (V) or constant (C) domain of Ig molecules. Members of the Ig superfamily include, but are not
30 limited to, the immunoglobulins themselves, major histocompatibility complex (MHC) class molecules (i.e., MHC class I and II), and TCR molecules.

In 1991, Linsley et al. *J. Exp. Med.* 174:561-569 (1991), proposed that CTLA-4 was a second receptor for B7. Similarly, Harper et al. *J Immunol* 147:1037-44 (1991) demonstrated that the CTLA-4 and CD28 molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. See also Balzano et al. *Int J Cancer Suppl* 7:28-32 (1992). Further evidence of this role arose through functional studies. For example, Lenschow et al. *Science* 257:789-792 (1992) demonstrated that CTLA-4-Ig induced long term survival of pancreatic islet grafts. Freeman et al. *Science* 262:907-909 (1993) examined the role of CTLA-4 in B7 deficient mice. Examination of the ligands for CTLA-4 are described in Lenschow et al. *P.N.A.S.* 90:11054-11058 (1993). Linsley et al. *Science* 257:792-795 (1992) describes immunosuppression *in vivo* by a soluble form of CTLA-4. Linsley et al. *J Exp Med* 176:1595-604 (1992) prepared antibodies that bound CTLA-4 and that were not cross-reactive with CD28 and concluded that CTLA-4 is coexpressed with CD28 on activated T lymphocytes and cooperatively regulates T cell adhesion and activation by B7. Kuchroo et al. *Cell* 80:707-18 (1995) demonstrated that the B7-1 and B7-2 costimulatory molecules differentially activated the Th1/Th2 developmental pathways. Yiqun et al. *Int Immunol* 8:37-44 (1996) demonstrated that there are differential requirements for co-stimulatory signals from B7 family members by resting versus recently activated memory T cells towards soluble recall antigens. See also de Boer et al. *Eur J Immunol* 23:3120-5 (1993).

Several groups proposed alternative or distinct receptor/ligand interactions for CTLA-4 as compared to CD28 and even proposed a third B-7 complex that was recognized by a BB1 antibody. See, for example, Hathcock et al. *Science* 262:905-7 (1993), Freeman et al. *Science* 262:907-9 (1993), Freeman et al. *J Exp Med* 178:2185-92 (1993), Lenschow et al. *Proc Natl Acad Sci U S A* 90:11054-8 (1993), Razi-Wolf et al. *Proc Natl Acad Sci U S A* 90:11182-6 (1993), and Boussiotis et al. *Proc Natl Acad Sci U S A* 90:11059-63 (1993). But, see, Freeman et al. *J Immunol* 161:2708-15 (1998) who discuss finding that BB1 antibody binds a molecule that is identical to the cell surface form of CD74 and, therefore, the BB1 mAb binds to a protein distinct from B7-

1, and this epitope is also present on the B7-1 protein. Thus, this observation required the field to reconsider studies using BB1 mAb in the analysis of CD80 expression and function.

Beginning in 1993 and culminating in 1995, investigators began to
5 further delineate the role of CTLA-4 in T-cell stimulation. First, through the use of monoclonal antibodies against CTLA-4, Walunas et al. *Immunity* 1:405-13 (1994) provided evidence that CTLA-4 can function as a negative regulator of T cell activation. Thereafter, Waterhouse et al. *Science* 270:985-988 (1995) demonstrated that mice deficient for CTLA-4 accumulated T cell blasts with up-
10 regulated activation markers in their lymph nodes and spleens. The blast cells also infiltrated liver, heart, lung, and pancreas tissue, and amounts of serum immunoglobulin were elevated and their T cells proliferated spontaneously and strongly when stimulated through the T cell receptor, however, they were sensitive to cell death induced by cross-linking of the Fas receptor and by
15 gamma irradiation. Waterhouse et al. concluded that CTLA-4 acts as a negative regulator of T cell activation and is vital for the control of lymphocyte homeostasis. In a comment in the same issue, Allison and Krummel *Science* 270:932-933 (1995), discussed the work of Waterhouse et al. as demonstrative that CTLA-4 acts to down regulate T-cell responsiveness or has an inhibitory
20 signaling role in T-cell activation and development. Tivol et al. *Immunity* 3:541-7 (1995) also generated CTLA-4-deficient mice and demonstrated that such mice rapidly develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis. They concluded that CTLA-4 plays a key role in down-regulating
25 T cell activation and maintaining immunologic homeostasis. Also, Krummel and Allison *J Exp Med* 182:459-65 (1995) further clarified that CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. They generated an antibody to CTLA-4 and investigated the effects of its binding to CTLA-4 in a system using highly purified T cells. In their report, they showed
30 that the presence of low levels of B7-2 on freshly explanted T cells can partially inhibit T cell proliferation, and this inhibition was mediated by interactions with CTLA-4. Cross-linking of CTLA-4 together with the TCR and CD28 strongly

inhibits proliferation and IL-2 secretion by T cells. Finally, the results showed that CD28 and CTLA-4 deliver opposing signals that appear to be integrated by the T cell in determining the response to antigen. Thus, they concluded that the outcome of T cell antigen receptor stimulation is regulated by CD28
5 costimulatory signals, as well as inhibitory signals derived from CTLA-4. *See also* Krummel et al. *Int Immunol* 8:519-23 (1996) and U.S. Patent No. 5,811,097 and International Patent Application No. WO 97/20574.

A variety of additional experiments have been conducted further elucidating the above function of CTLA-4. For example, Walunas et al. *J Exp*
10 *Med* 183:2541-50 (1996), through the use of anti-CTLA-4 antibodies, suggested that CTLA-4 signaling does not regulate cell survival or responsiveness to IL-2, but does inhibit CD28-dependent IL-2 production. Also, Perrin et al. *J Immunol* 157:1333-6 (1996), demonstrated that anti-CTLA-4 antibodies in experimental allergic encephalomyelitis (EAE), exacerbated the disease and enhanced
15 mortality. Disease exacerbation was associated with enhanced production of the encephalitogenic cytokines TNF-alpha, IFN-gamma and IL-2. Thus, they concluded that CTLA-4 regulates the intensity of the autoimmune response in EAE, attenuating inflammatory cytokine production and clinical disease manifestations. *See also* Hurwitz et al. *J Neuroimmunol* 73:57-62 (1997) and
20 Cepero et al. *J Exp Med* 188:199-204 (1998) (an anti-CTLA-4 hairpin ribozyme that specifically abrogates CTLA-4 expression after gene transfer into a murine T-cell model).

In addition, Blair et al. *J Immunol* 160:12-5 (1998) assessed the functional effects of a panel of CTLA-4 monoclonal antibodies (mAbs) on
25 resting human CD4+ T cells. Their results demonstrated that some CTLA-4 mAbs could inhibit proliferative responses of resting CD4+ cells and cell cycle transition from G0 to G1. The inhibitory effects of CTLA-4 were evident within 4 h, at a time when cell surface CTLA-4 expression remained undetectable. Other CTLA-4 mAbs, however, had no detectable inhibitory effects, indicating
30 that binding of mAbs to CTLA-4 alone was not sufficient to mediate down-regulation of T cell responses. Interestingly, while IL-2 production was shut off, inhibitory anti-CTLA-4 mAbs permitted induction and expression of the cell

survival gene bcl-X(L). Consistent with this observation, cells remained viable and apoptosis was not detected after CTLA-4 ligation.

In connection with anergy, Perez et al. *Immunity* 6:411-7 (1997) demonstrated that the induction of T cell anergy was prevented by blocking CTLA-4 and concluded that the outcome of antigen recognition by T cells is determined by the interaction of CD28 or CTLA-4 on the T cells with B7 molecules. Also, Van Parijs et al. *J Exp Med* 186:1119-28 (1997) examined the role of interleukin 12 and costimulators in T cell anergy *in vivo* and found that through inhibiting CTLA-4 engagement during anergy induction, T cell proliferation was blocked, and full Th1 differentiation was not promoted. However, T cells exposed to tolerogenic antigen in the presence of both IL-12 and anti-CTLA-4 antibody were not anergized, and behaved identically to T cells which have encountered immunogenic antigen. These results suggested that two processes contribute to the induction of anergy *in vivo*: CTLA-4 engagement, which leads to a block in the ability of T cells to proliferate, and the absence of a prototypic inflammatory cytokine, IL-12, which prevents the differentiation of T cells into Th1 effector cells. The combination of IL-12 and anti-CTLA-4 antibody was sufficient to convert a normally tolerogenic stimulus to an immunogenic one.

In connection with infections, McCoy et al. *J Exp Med* 186:183-7 (1997) demonstrated that anti-CTLA-4 antibodies greatly enhanced and accelerated the T cell immune response to *Nippostrongylus brasiliensis*, resulting in a profound reduction in adult worm numbers and early termination of parasite egg production. See also Murphy et al. *J. Immunol.* 161:4153-4160 (1998) (*Leishmania donovani*).

In connection with cancer, Kwon et al. *PNAS USA* 94:8099-103 (1997) established a syngeneic murine prostate cancer model and examined two distinct manipulations intended to elicit an antiprostata cancer response through enhanced T cell costimulation: (i) provision of direct costimulation by prostate cancer cells transduced to express the B7.1 ligand and (ii) *in vivo* antibody-mediated blockade of T cell CTLA-4, which prevents T cell down-regulation. It was demonstrated that *in vivo* antibody-mediated blockade of CTLA-4

enhanced antiprostata cancer immune responses. Also, Yang et al. *Cancer Res* 57:4036-41 (1997) investigated whether the blockade of the CTLA-4 function leads to enhancement of antitumor T cell responses at various stages of tumor growth. Based on *in vitro* and *in vivo* results they found that CTLA-4 blockade
5 in tumor-bearing individuals enhanced the capacity to generate antitumor T-cell responses, but the expression of such an enhancing effect was restricted to early stages of tumor growth in their model. Further, Hurwitz et al. *Proc Natl Acad Sci U S A* 95:10067-71 (1998) investigated the generation of a T cell-mediated antitumor response depends on T cell receptor engagement by major
10 histocompatibility complex/antigen as well as CD28 ligation by B7. Certain tumors, such as the SM1 mammary carcinoma, were refractory to anti-CTLA-4 immunotherapy. Thus, through use of a combination of CTLA-4 blockade and a vaccine consisting of granulocyte-macrophage colony-stimulating factor-expressing SM1 cells, regression of parental SM1 tumors was observed, despite
15 the ineffectiveness of either treatment alone. This combination therapy resulted in long-lasting immunity to SM1 and depended on both CD4(+) and CD8(+) T cells. The findings suggested that CTLA-4 blockade acts at the level of a host-derived antigen-presenting cell.

In connection with diabetes, Luhder et al. *J Exp Med* 187:427-32 (1998)
20 injected an anti-CTLA-4 mAb into a TCR transgenic mouse model of diabetes at different stages of disease. They found that engagement of CTLA-4 at the time when potentially diabetogenic T cells are first activated is a pivotal event; if engagement is permitted, invasion of the islets occurs, but remains quite innocuous for months. If not, insulitis is much more aggressive, and diabetes
25 quickly ensues.

In connection with vaccine immunization, Horspool et al. *J Immunol* 160:2706-14 (1998) found that intact anti-CTLA-4 mAb but not Fab fragments suppressed the primary humoral response to pCIA/beta gal without affecting recall responses, indicating CTLA-4 activation inhibited Ab production but not
30 T cell priming. Blockade of the ligands for CD28 and CTLA-4, CD80 (B7-1) and CD86 (B7-2), revealed distinct and nonoverlapping function. Blockade of CD80 at initial immunization completely abrogated primary and secondary Ab

responses, whereas blockade of CD86 suppressed primary but not secondary responses. Simultaneous blockade of CD80 + CD86 was less effective at suppressing Ab responses than either alone. Enhancement of costimulation via coinjection of B7-expressing plasmids augmented CTL responses but not Ab responses, and without evidence of Th1 to Th2 skewing. These findings suggest complex and distinct roles for CD28, CTLA-4, CD80, and CD86 in T cell costimulation following nucleic acid vaccination.

In connection with allograft rejection, Markees et al. *J Clin Invest* 101:2446-55 (1998) found in a mouse model of skin allograft rejection that acceptance initially depended on the presence of IFN-gamma, CTLA-4, and CD4(+) T cells. Addition of anti-CTLA-4 or anti-IFN-gamma mAb to the protocol was associated with prompt graft rejection, whereas anti-IL-4 mAb had no effect.

In connection with the role of CTLA-4 in relation to CD28, Fallarino et al. *J Exp Med* 188:205-10 (1998) generated TCR transgenic/recombinase activating gene 2-deficient/CD28-wild-type or CD28-deficient mice which were immunized with an antigen-expressing tumor. Primed T cells from both types of mice produced cytokines and proliferated in response to stimulator cells lacking B7 expression. However, whereas the response of CD28+/+ T cells was augmented by costimulation with B7-1, the response of the CD28-/- T cells was strongly inhibited. This inhibition was reversed by monoclonal antibody against B7-1 or CTLA-4. Thus, CTLA-4 can potentially inhibit T cell activation in the absence of CD28, indicating that antagonism of a TCR-mediated signal is sufficient to explain the inhibitory effect of CTLA-4. Also, Lin et al. *J Exp Med* 188:199-204 (1998) studied rejection of heart allografts in CD28-deficient mice. H-2(q) hearts were transplanted into allogeneic wild-type or CD28-deficient mice (H-2(b)). Graft rejection was delayed in CD28-deficient compared with wild-type mice. Treatment of wild-type recipients with CTLA-4-immunoglobulin (Ig), or with anti-B7-1 plus anti-B7-2 mAbs significantly prolonged allograft survival. In contrast, treatment of CD28-deficient mice with CTLA-4-Ig, anti-B7-1 plus anti-B7-2 mAbs, or a blocking anti-CTLA-4 mAb induced acceleration of allograft rejection. This increased rate of graft rejection

was associated with more severe mononuclear cell infiltration and enhanced levels of IFN-gamma and IL-6 transcripts in donor hearts of untreated wild-type and CTLA-4-Ig- or anti-CTLA-4 mAb-treated CD28-deficient mice. Thus, the negative regulatory role of CTLA-4 extends beyond its potential ability to prevent CD28 activation through ligand competition. Even in the absence of CD28, CTLA-4 plays an inhibitory role in the regulation of allograft rejection.

Also, further characterization of the expression of CTLA-4 has been investigated. For example, Alegre et al. *J Immunol* 157:4762-70 (1996) proposed that surface CTLA-4 is rapidly internalized, which may explain the low levels of expression generally detected on the cell surface. They concluded that both CD28 and IL-2 play important roles in the up-regulation of CTLA-4 expression. In addition, the cell surface accumulation of CTLA-4 appeared to be primarily regulated by its rapid endocytosis. Also, Castan et al. *Immunology* 90:265-71 (1997) based on *in situ* immunohistological analyses of the expression of CTLA-4, suggested that germinal center T cells, which were CTLA-4 positive, could be important to immune regulation.

Accordingly, in view of the broad and pivotal role that CTLA-4 appears to possess in immune responsiveness, it would be desirable to generate antibodies to CTLA-4 that can be utilized effectively in immunotherapy. Moreover, it would be desirable to generate antibodies against CTLA-4 that can be utilized in chronic diseases in which repeat administrations of the antibodies are required.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

Figure 1 provides a series of nucleotide and an amino acid sequences of heavy chain and kappa light chain immunoglobulin molecules in accordance with the invention: 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M).

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1,

4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "0s".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17

amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

5 Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as
10 underlined.

Figure 9 provides a summary of N-terminal amino acid sequences generated through direct protein sequencing of the heavy and light chains of the antibodies.

Figure 10 provides certain additional characterizing information about
15 certain of the antibodies in accordance with the invention. In Figure 10A, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. Data related to concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, liquid chromatography/mass spectroscopy (LCMS), mass spectroscopy (MALDI), light chain N-terminal sequences is provided.
20 Additional detailed information related to IEF is provided in Figure 10B; related to SDS-PAGE is provided in 10C; and SEC of the 4.1.1 antibody in 10D.

Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs.

Figure 12 shows the concentration dependent enhancement of IL-2
25 production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1).

Figure 13 shows the concentration dependent enhancement of IFN- γ production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1)(same donor T cells).

30 Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 15 shows the mean enhancement of IFN- γ production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 16 shows the enhancement of IL-2 production in hPBMC from 5 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 hours after stimulation with SEA.

Figure 17 shows the enhancement of IL-2 production in whole blood from 3 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 and 96 hours after stimulation with SEA.

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model.

Figure 19 shows enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji and Superantigen (whole blood and peripheral blood mononuclear cells from 6 donors) assays.

Figure 20 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji assay.

Figure 21 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour Superantigen whole blood assay stimulated with 100 ng/ml superantigen.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains: full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c)), full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e)), 4.1.1 light chain (cDNA 22(f) and amino acid 22(g)), full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i)), 4.8.1 light chain (cDNA 22(j) and amino acid 22(k)), full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m)), 6.1.1 light chain (cDNA 22(n) and amino acid 22(o)), full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)), and 11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided an antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V_H3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or framework sequences shown in Figure 2. In a preferred embodiment, the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70. In another preferred embodiment, the antibody further comprises a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

In accordance with a second aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:1 and a light chain variable amino acid sequence comprising SEQ ID NO:14.

In accordance with a third aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:2 and a light chain variable amino acid sequence comprising SEQ ID NO:15.

In accordance with a fourth aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:4 and a light chain variable amino acid sequence comprising SEQ ID NO:17.

In accordance with a fifth aspect of the present invention, there is provided an isolated human monoclonal antibody that is capable of binding to CTLA-4. In a preferred embodiment, antibody is capable of competing for binding with CTLA-4 with an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian species comprises mouse, rat, and rabbit and more preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2

production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a sixth aspect of the present invention, there is
5 provided a humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1.1, 4.1.1.1, 4.8.1.1, 4.10.2.1, 4.13.1.1, 4.14.3.1, 6.1.1.1, 11.2.1.1, 11.6.1.1, 11.7.1.1, 12.3.1.1.1, and 12.9.1.1.1. In a preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian
10 species comprises mouse, rat, and rabbit and preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and
15 preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the
20 antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN-
25 γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the
30 antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a seventh aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2. In a preferred embodiment, the antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

In accordance with an eighth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties: a binding affinity for CTLA-4 of about 10⁻⁹ or greater; inhibits binding between CTLA-4 and B7-1 with an IC₅₀ of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC₅₀ of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a ninth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties: a binding affinity for CTLA-4 of about 10⁻⁹ or greater; inhibits binding between CTLA-4 and B7-1 with an IC₅₀ of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC₅₀ of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a tenth aspect of the present invention, there is provided a cell culture system for assaying T cell stimulation, comprising a culture of human T cell blasts co-cultured with a Raji cell line. In a preferred embodiment, the T cell blasts are washed prior to culture with the Raji cell line.

In accordance with an eleventh aspect of the present invention, there is provided an assay for measuring T cell stimulation, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with an agent; and measuring cytokine production by the culture.

5 In accordance with an twelfth aspect of the present invention, there is provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with the moiety; and assessing cytokine production by the culture.

10 In accordance with a thirteenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

In accordance with a fourteenth aspect of the present invention, there is
15 provided a method for screening an agent for T cell stimulatory activity, comprising: contacting the agent with a cell culture comprising human T cell blasts and a Raji cell line; and assessing cytokine production by the culture.

In each of the tenth through the fourteenth aspects of the present invention, in a preferred embodiment, the T cell blasts are washed prior to
20 culture with the Raji cell line. In another preferred embodiment, the cytokine is IL-2 or IFN- γ . In a preferred embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

In accordance with a fifteenth aspect of the present invention, there is
25 provided an assay for measuring T cell stimulation, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; contacting the culture with an agent; and measuring cytokine production by the cell population.

In accordance with a sixteenth aspect of the present invention, there is
30 provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus

enterotoxin A; contacting the culture with the moiety; and assessing cytokine production by the cell population.

In accordance with a seventeenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising
5 contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.

In accordance with an eighteenth aspect of the present invention, there is provided a method for screening an agent for T cell stimulatory activity,
10 comprising: contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and assessing cytokine production by the cell population.

In each of the fifteenth through the eighteenth aspects of the present invention, in a preferred embodiment, the cytokine is IL-2. In another preferred
15 embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20

In accordance with the present invention, there are provided fully human monoclonal antibodies against human CTLA-4. Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly sequences corresponding to a contiguous heavy and light
25 chain sequences from FR1 and CDR1 through CDR3 and FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

30

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

The term "polypeptide" as used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules and the human kappa light chain immunoglobulin molecules represented in Figure 1, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant.

Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem. Soc.* 106:6077 (1984); Stein et al. *Nucl. Acids Res.* 16:3209 (1988); Zon et al. *Anti-Cancer Drug Design* 6:539 (1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for

maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff, M.O., in *Atlas of Protein Sequence and Structure*, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1)

comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both

sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the

amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains.

- 5 Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are
- 10 aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or
- 15 a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide
- 20 derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid
- 25 sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. *Science* 253:164
- 30 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each incorporated herein by reference.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to CTLA-4, under suitable

binding conditions, (2) ability to block CTLA-4 binding with its receptors, or (3) ability to inhibit CTLA-4 expressing cell growth *in vitro* or *in vivo*. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence.

5 Analogues typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

Peptide analogs are commonly used in the pharmaceutical industry as

10 non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p.392 (1985); and Evans et al. *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often

15 developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such

20 as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH₂NH--, --CH₂S--, --CH₂-CH₂--, --CH=CH--(cis and trans), --COCH₂--, --CH(OH)CH₂--, and --CH₂SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same

25 type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal

30 cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments
5 include Fab, Fab', F(ab')₂, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60% or 80%, and more
10 usually greater than about 85% (as measured in an *in vitro* competitive binding assay).

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually
15 consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ and most preferably $\leq 10 \text{ nM}$.

20

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

25 As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations,
30 the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the

following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, 5 predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

10 The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San 15 Francisco (1985)), incorporated herein by reference).

The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a 20 carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any 25 other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more 30 preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot

be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term patient includes human and veterinary subjects.

5

Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions

of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody
5 having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies"
10 (Holliger et al. "Diabodies": small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).
15 Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

20

Human Antibodies and Humanization of Antibodies

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The
25 presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, it has been postulated that one can develop humanized antibodies or generate fully human antibodies through the introduction of
30 human antibody function into a rodent so that the rodent would produce antibodies having fully human sequences.

Human Antibodies

The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance

to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and
5 selected.

This general strategy was demonstrated in connection with our generation of the first XenoMouse™ strains as published in 1994. *See* Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and
10 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes.
15 This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might
20 recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light
25 chain loci, respectively, to produce XenoMouse™ mice. *See* Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

30 Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30,

1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 5 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998). *See also* European Patent No., EP 0 463 151 B1, grant published 10 June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

15 In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region 20 (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Patent No. 5,545,807 to Surani et al. and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, and 5,814,318 each to Lonberg and Kay, U.S. Patent No. 5,591,669 to Krimpenfort and Berns, U.S. Patent Nos. 5,612,205, 25 5,721,367, 5,789,215 to Berns et al., and U.S. Patent No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. Patent Application Serial Nos. 07/574,748, filed August 29, 1990, 07/575,962, filed August 31, 1990, 07/810,279, filed December 17, 1991, 07/853,408, filed March 18, 1992, 07/904,068, filed June 23, 1992, 07/990,860, filed December 16, 1992, 30 08/053,131, filed April 26, 1993, 08/096,762, filed July 22, 1993, 08/155,301, filed November 18, 1993, 08/161,739, filed December 3, 1993, 08/165,699, filed December 10, 1993, 08/209,741, filed March 9, 1994, the disclosures of

which are hereby incorporated by reference. *See also* European Patent No. 0 546 073 B1, International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of which are hereby incorporated by reference in their entirety. *See further* Taylor et al., 1992, Chen et al., 1993, Tuailon et al., 1993, Choi et al., 1993, Lonberg et al., (1994), Taylor et al., (1994), and Tuailon et al., (1995), Fishwild et al., (1996), the disclosures of which are hereby incorporated by reference in their entirety.

10 The inventors of Surani et al., cited above and assigned to the Medical Research Counsel (the "MRC"), produced a transgenic mouse possessing an Ig locus through use of the minilocus approach. The inventors on the GenPharm International work, cited above, Lonberg and Kay, following the lead of the present inventors, proposed inactivation of the endogenous mouse Ig locus
15 coupled with substantial duplication of the Surani et al. work.

 An advantage of the minilocus approach is the rapidity with which constructs including portions of the Ig locus can be generated and introduced into animals. Commensurately, however, a significant disadvantage of the minilocus approach is that, in theory, insufficient diversity is introduced through
20 the inclusion of small numbers of V, D, and J genes. Indeed, the published work appears to support this concern. B-cell development and antibody production of animals produced through use of the minilocus approach appear stunted. Therefore, research surrounding the present invention has consistently been directed towards the introduction of large portions of the Ig locus in order
25 to achieve greater diversity and in an effort to reconstitute the immune repertoire of the animals.

 Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is
30 expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody.

Thus, it would be desirable to provide fully human antibodies against CTLA-4 in order to vitiate concerns and/or effects of HAMA or HACA response.

Humanization and Display Technologies

5 As was discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. To a degree, this can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be
10 humanized or primatized using techniques well known in the art. See e.g., Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12:125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human
15 sequence (see WO 92/02190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al. *P.N.A.S.* 84:3439 (1987) and *J.Immunol.* 139:3521 (1987)). mRNA is isolated
20 from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to
25 human constant region sequences. The sequences of human constant regions genes may be found in Kabat et al. (1991) *Sequences of Proteins of Immunological Interest*, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in
30 antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG2, IgG3 and IgG4. Particularly preferred isotypes for antibodies of the invention are IgG2 and IgG4. Either of the human light chain constant regions, kappa or

lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

Antibody fragments, such as Fv, F(ab')₂ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage.

- 5 Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

- 10 In one approach, consensus sequences encoding the heavy and light chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

- 15 Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between
20 the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral
25 LTRs, e.g. SV-40 early promoter, (Okayama et al. *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al. *P.N.A.S.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al. *Cell* 41:885 (1985)); native Ig promoters, etc.

- 30 Further, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to

additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, *supra.*, Hanes and Plutchau *PNAS USA* 94:4937-4942 (1997) (ribosomal display), Parmley and Smith *Gene* 73:305-318 (1988) (phage display), Scott *TIBS* 17:241-245 (1992), Cwirla et al. *PNAS USA* 87:6378-6382 (1990), Russel et al. *Nucl. Acids Research* 21:1081-1085 (1993), Hoganboom et al. *Immunol. Reviews* 130:43-68 (1992), Chiswell and McCafferty *TIBTECH* 10:80-84 (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

Using these techniques, antibodies can be generated to CTLA-4 expressing cells, CTLA-4 itself, forms of CTLA-4, epitopes or peptides thereof, and expression libraries thereto (*see e.g.* U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described above.

Additional Criteria for Antibody Therapeutics

As will be appreciated, it is generally not desirable to kill CTLA-4 expressing cells. Rather, one generally desires to simply inhibit CTLA-4 binding with its ligands to mitigate T cell down regulation. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. Those isotypes that do not include, without limitation, human IgG2 and human IgG4.

It will be appreciated that antibodies that are generated need not initially possess a particular desired isotype but, rather, the antibody as generated can

possess any isotype and the antibody can be isotype switched thereafter using conventional techniques that are well known in the art. Such techniques include the use of direct recombinant techniques (*see e.g.*, U.S. Patent No. 4,816,397), cell-cell fusion techniques (*see e.g.*, U.S. Patent Application No. 08/730,639, filed October 11, 1996), among others.

In the cell-cell fusion technique, a myeloma or other cell line is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line is prepared that possesses the light chain. Such cells can, thereafter, be fused and a cell line expressing an intact antibody can be isolated.

By way of example, the majority of the CTLA-4 antibodies discussed herein are human anti-CTLA-4 IgG2 antibody. Since such antibodies possess desired binding to the CTLA-4 molecule, any one of such antibodies can be readily isotype switched to generate a human IgG4 isotype, for example, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity).

Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain additional "functional" attributes that are desired through isotype switching.

Design and Generation of Other Therapeutics

In accordance with the present invention and based on the activity of the antibodies that are produced and characterized herein with respect to CTLA-4, the design of other therapeutic modalities including other antibodies, other antagonists, or chemical moieties other than antibodies is facilitated. Such modalities include, without limitation, antibodies having similar binding activity or functionality, advanced antibody therapeutics, such as bispecific antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules. Furthermore, as discussed above, the effector function of the

antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses.

In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to
5 sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels, for example.

In connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies one with a specificity to CTLA-4 and another to a second molecule that are conjugated together, (ii) a single antibody
10 that has one chain specific to CTLA-4 and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to CTLA-4 and the other molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) *see e.g.*, Fanger et al. *Immunol Methods* 4:72-81 (1994) and Wright and Harris, *supra.* and in
15 connection with (iii) *see e.g.*, Traunecker et al. *Int. J. Cancer (Suppl.)* 7:51-52 (1992).

In addition, "Kappabodies" (Ill et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" *Protein Eng* 10:949-57 (1997)), "Minibodies" (Martin et al.
20 "The affinity-selection of a minibody polypeptide inhibitor of human interleukin-6" *EMBO J* 13:5303-9 (1994)), "Diabodies" (Holliger et al. "Diabodies": small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)), or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells"
25 *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)) may also be prepared.

In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. *See e.g.*,
30 Vitetta *Immunol Today* 14:252 (1993). *See also* U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known

in the art. See e.g., Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). See also U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902. Each of immunotoxins and radiolabeled molecules
5 would be likely to kill cells expressing CTLA-4, and particularly those cells in which the antibodies of the invention are effective.

In connection with the generation of therapeutic peptides, through the utilization of structural information related to CTLA-4 and antibodies thereto, such as the antibodies of the invention (as discussed below in connection with
10 small molecules) or screening of peptide libraries, therapeutic peptides can be generated that are directed against CTLA-4. Design and screening of peptide therapeutics is discussed in connection with Houghten et al. *Biotechniques* 13:412-421 (1992), Houghten *PNAS USA* 82:5131-5135 (1985), Pinalla et al. *Biotechniques* 13:901-905 (1992), Blake and Litzi-Davis *BioConjugate Chem.*
15 3:510-513 (1992). Immunotoxins and radiolabeled molecules can also be prepared, and in a similar manner, in connection with peptidic moieties as discussed above in connection with antibodies.

Important information related to the binding of an antibody to an antigen can be gleaned through phage display experimentation. Such experiments are
20 generally accomplished through panning a phage library expressing random peptides for binding with the antibodies of the invention to determine if peptides can be isolated that bind. If successful, certain epitope information can be gleaned from the peptides that bind.

In general, phage libraries expressing random peptides can be purchased
25 from New England Biolabs (7-mer and 12-mer libraries, Ph.D.-7 Peptide 7-mer Library Kit and Ph.D.-12 Peptide 12-mer Library Kit, respectively) based on a bacteriophage M13 system. The 7-mer library represents a diversity of approximately 2.0×10^9 independent clones, which represents most, if not all, of the $20^7 = 1.28 \times 10^9$ possible 7-mer sequences. The 12-mer library contains
30 approximately 1.9×10^9 independent clones and represents only a very small sampling of the potential sequence space of $20^{12} = 4.1 \times 10^{15}$ 12-mer sequences. Each of 7-mer and 12-mer libraries are panned or screened in accordance with

the manufacturer's recommendations in which plates were coated with an antibody to capture the appropriate antibody (a goat anti-human IgG Fc for an IgG antibody for example) followed by washing. Bound phage are eluted with 0.2 M glycine-HCl, pH 2.2. After 3 rounds of selection/amplification at
5 constant stringency (0.5% Tween), through use of DNA sequencing, one can characterize clones from the libraries that are reactive with one or more of the antibodies. Reactivity of the peptides can be determined by ELISA. For an additional discussion of epitope analysis of peptides *see also* Scott, J.K. and Smith, G.P. *Science* 249:386-390 (1990); Cwirla et al. *PNAS USA* 87:6378-
10 6382 (1990); Felici et al. *J. Mol. Biol.* 222:301-310 (1991), and Kuwabara et al. *Nature Biotechnology* 15:74-78 (1997).

The design of gene and/or antisense therapeutics through conventional techniques is also facilitated through the present invention. Such modalities can be utilized for modulating the function of CTLA-4. In connection therewith the
15 antibodies of the present invention facilitate design and use of functional assays related thereto. A design and strategy for antisense therapeutics is discussed in detail in International Patent Application No. WO 94/29444. Design and strategies for gene therapy are well known. However, in particular, the use of gene therapeutic techniques involving intrabodies could prove to be particularly
20 advantageous. *See e.g.*, Chen et al. *Human Gene Therapy* 5:595-601 (1994) and Marasco *Gene Therapy* 4:11-15 (1997). General design of and considerations related to gene therapeutics is also discussed in International Patent Application No. WO 97/38137. Genetic materials encoding an antibody of the invention (such as the 4.1.1, 4.8.1, or 6.1.1, or others) may be included in a suitable
25 expression system (whether viral, attenuated viral, non-viral, naked, or otherwise) and administered to a host for *in vivo* generation of the antibody in the host.

Small molecule therapeutics can also be envisioned in accordance with the present invention. Drugs can be designed to modulate the activity of CTLA-
30 4 based upon the present invention. Knowledge gleaned from the structure of the CTLA-4 molecule and its interactions with other molecules in accordance with the present invention, such as the antibodies of the invention, CD28, B7,

B7-1, B7-2, and others can be utilized to rationally design additional therapeutic modalities. In this regard, rational drug design techniques such as X-ray crystallography, computer-aided (or assisted) molecular modeling (CAMP), quantitative or qualitative structure-activity relationship (QSAR), and similar technologies can be utilized to focus drug discovery efforts. Rational design allows prediction of protein or synthetic structures which can interact with the molecule or specific forms thereof which can be used to modify or modulate the activity of CTLA-4. Such structures can be synthesized chemically or expressed in biological systems. This approach has been reviewed in Capsey et al. *Genetically Engineered Human Therapeutic Drugs* (Stockton Press, NY (1988)). Indeed, the rational design of molecules (either peptides, peptidomimetics, small molecules, or the like) based upon known, or delineated, structure-activity relationships with other molecules (such as antibodies in accordance with the invention) has become generally routine. See, e.g., Fry et al. "Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor" *Proc Natl Acad Sci U S A* 95:12022-7 (1998); Hoffman et al. "A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese homology domain" *J Mol Biol* 282:195-208 (1998); Ginalski et al. "Modelling of active forms of protein kinases: p38--a case study" *Acta Biochim Pol* 44:557-64 (1997); Jouko et al. "Identification of csk tyrosine phosphorylation sites and a tyrosine residue important for kinase domain structure" *Biochem J* 322:927-35 (1997); Singh et al. "Structure-based design of a potent, selective, and irreversible inhibitor of the catalytic domain of the erbB receptor subfamily of protein tyrosine kinases" *J Med Chem* 40:1130-5 (1997); Mandel et al. "ABGEN: a knowledge-based automated approach for antibody structure modeling" *Nat Biotechnol* 14:323-8 (1996); Monfardini et al. "Rational design, analysis, and potential utility of GM-CSF antagonists" *Proc Assoc Am Physicians* 108:420-31 (1996); Furet et al. "Modelling study of protein kinase inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411" *J Comput Aided Mol Des* 9:465-72 (1995).

Further, combinatorial libraries can be designed and synthesized and used in screening programs, such as high throughput screening efforts.

Therapeutic Administration and Formulations

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It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LipofectinTM), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

25

Preparation of Antibodies

Antibodies in accordance with the invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of

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producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the Background, herein. In particular, however, a preferred embodiment of transgenic production of mice and antibodies therefrom is disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosure of which is hereby incorporated by reference. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997), the disclosure of which is hereby incorporated by reference.

Through use of such technology, we have produced fully human monoclonal antibodies to a variety of antigens. Essentially, we immunize XenoMouse™ lines of mice with an antigen of interest, recover lymphatic cells (such as B-cells) from the mice that express antibodies, fuse such recovered cells with a myeloid-type cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. We utilized these techniques in accordance with the present invention for the preparation of antibodies specific to CTLA-4. Herein, we describe the production of multiple hybridoma cell lines that produce antibodies specific to CTLA-4. Further, we provide a characterization of the antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

The antibodies derived from hybridoma cell lines discussed herein are designated 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. Each of the antibodies produced by the aforementioned cell lines are either fully human IgG2 or IgG4 heavy chains with human kappa light chains. In general, antibodies in accordance with the invention possess very high affinities, typically possessing K_d 's of from about 10^{-9} through about 10^{-11} M, when measured by either solid phase or solution phase.

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines.

Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed.

10 Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct

15 microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO₀, HeLa cells, baby hamster kidney (BHK) cells,

20 monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either

25 the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive CTLA-4 binding properties.

Further, expression of antibodies of the invention (or other moieties

30 therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase and DHFR gene expression systems are common approaches for enhancing expression under certain

conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 5 89303964.4.

Antibodies of the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in 10 mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. *See, e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

Antibodies in accordance with the present invention have been analyzed structurally and functionally. In connection with the structures of the 15 antibodies, amino acid sequences of the heavy and kappa light chains have been predicted based on cDNA sequences obtained through RT-PCR of the hybridomas. *See* Examples 3 and 4 and Figures 1-8. N-terminal sequencing of the antibodies was also conducted in confirmation of the results discussed in Examples 3 and 4. *See* Example 5 and Figure 9. Kinetic analyses of the 20 antibodies were conducted to determine affinities. *See* Example 2. Antibodies in accordance with the invention (and particularly the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) have high affinities ($4.1.1:1.63 \times 10^{10}$ 1/M; $4.8.1:3.54 \times 10^{10}$ 1/M; and $6.1.1:7.2 \times 10^9$ 1/M). Further, antibodies were analyzed by isoelectric focusing (IEF), reducing gel electrophoresis (SDS- 25 PAGE), size exclusion chromatography, liquid chromatography/mass spectroscopy, and mass spectroscopy and antibody production by the hybridomas was assessed. *See* Example 6 and Figure 10.

In connection with functional analysis of antibodies in accordance with the present invention, such antibodies proved to be potent inhibitors of CTLA-4 30 and its binding to its ligands of the B7 family of molecules. For example, antibodies in accordance with the present invention were demonstrated to block CTLA-4 binding to either B7-1 or B7-2. *See* Example 7. Indeed, many of the

antibodies in accordance with the invention possess nanomolar and subnanomolar IC_{50} s with respect to inhibiting CTLA-4 binding to B7-1 and B7-2. Further, antibodies of the invention possess excellent selectivity for CTLA-4 as compared to CD28, CD44, B7-2, or hIgG1. *See* Example 8. Selectivity is a ratio that reflects the degree of preferential binding of a molecule with a first agent as compared to the molecules binding with a second, and optionally other molecules. Herein, selectivity refers to the degree of preferential binding of an antibody of the invention to CTLA-4 as compared to the antibody's binding to other molecules such as CD28, CD44, B7-2, or hIgG1. Selectivity values of antibodies of the invention greater than 500:1 are common. Antibodies of the invention have also been demonstrated to induce or enhance expression of certain cytokines (such as IL-2 and IFN- γ) by cultured T cells in a T cell blast model. *See* Examples 9 and 10 and Figures 12-17. Further, it is expected that antibodies of the invention will inhibit the growth of tumors in appropriate *in vivo* tumor models. The design of which models are discussed in Example 11 and 12.

The results demonstrated in accordance with the present invention indicate that antibodies of the present invention possess certain qualities that may make the present antibodies more efficacious than current therapeutic antibodies against CTLA-4.

In particular, the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention possess highly desirable properties. Their structural characteristics, functions, or activities provide criteria that facilitate the design or selection of additional antibodies or other molecules as discussed above. Such criteria include one or more of the following:

Ability to compete for binding to CTLA-4 with one or more of the antibodies of the invention;

Similar binding specificity to CTLA-4 as one or more of the antibodies of the invention;

A binding affinity for CTLA-4 of about 10^{-9} M or greater and preferably of about 10^{-10} M or greater;

Does not cross react with lower mammalian CTLA-4, including, preferably, mouse, rat, or rabbit and preferably mouse or rat CTLA-4;

Cross reacts with primate CTLA-4, including, preferably, cynomolgous and rhesus CTLA-4;

5 A selectivity for CTLA-4 over CD28, B7-2, CD44, or hIgG1 of at least about 100:1 or greater and preferably of about 300, 400, or 500:1 or greater;

An IC_{50} in blocking CTLA-4 binding to B7-2 of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.38 nM or lower;

10 An IC_{50} in blocking CTLA-4 binding to B7-1 of about of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.50 nM or lower;

An enhancement of cytokine production in one or more *in vitro* assays, for example:

15 An enhancement of IL-2 production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, 1500, 2000, 3000, or 3846 pg/ml or greater;

An enhancement of IFN- γ production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, or 1233 pg/ml or greater; or

20 An enhancement of IL-2 production in a hPBMC or whole blood superantigen assay of about 500 pg/ml or greater and preferably 750, 1000, 1200, or 1511 pg/ml or greater. Expressed another way, it is desirable that IL-2 production is enhanced by about 30, 35, 40, 45, 50 percent or more relative to control in the assay.

25 It is expected that antibodies (or molecules designed or synthesized therefrom) having one or more of these properties will possess similar efficacy to the antibodies described in the present invention.

30 The desirable functional properties discussed above can often result from binding to and inhibition of CTLA4 by a molecule (i.e., antibody, antibody fragment, peptide, or small molecule) in a similar manner as an antibody of the invention (i.e., binding to the same or similar epitope of the CTLA4 molecule).

The molecule may either be administered directly (i.e., direct administration to a patient of such molecules). Or, alternatively, the molecule may be "administered" indirectly (i.e., a peptide or the like that produces an immune response in a patient (similar to a vaccine) wherein the immune response includes the generation of antibodies that bind to the same or similar epitope or an antibody or fragment that is produced *in situ* after administration of genetic materials that encode such antibodies or fragments thereof which bind to the same or similar epitope). Thus, it will be appreciated that the epitope on CTLA4 to which antibodies of the invention bind to can be useful in connection with the preparation and/or design of therapeutics in accordance with the invention. In drug design, negative information is often useful as well (i.e., the fact that an antibody which binds to CTLA4 does not appear to bind to an epitope that acts as an inhibitor of CTLA4 is useful). Thus, the epitope to which antibodies of the invention bind that do not lead to the desired functionality can also be very useful. Accordingly, also contemplated in accordance with the present invention are molecules (and particularly antibodies) that bind to the same or similar epitopes as antibodies of the invention.

In addition to the fact that antibodies of the invention and the epitopes to which they bind are contemplated in accordance with the invention, we have conducted some preliminary epitope mapping studies of certain antibodies in accordance with the invention and particularly the 4.1.1 and the 11.2.1 antibodies of the invention.

As a first step, we conducted BIAcore competition studies to generate a rough map of binding as between certain antibodies of the invention in connection with their ability to compete for binding to CTLA4. To this end, CTLA4 was bound to a BIAcore chip and a first antibody, under saturating conditions, was bound thereto and competition of subsequent secondary antibodies binding to CTLA4 was measured. This technique enabled generation of a rough map in to which families of antibodies can be classified.

Through this process, we determined that the certain antibodies in accordance with the invention could be categorized as falling into the following epitopic categories:

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Category	Antibodies	Competition for CTLA4 Binding
A	BO1M*	Freely cross-compete with one another; cross-compete with category B; some cross-competition with category D
	BO2M**	
B	4.1.1	Freely cross-compete with one another; cross-compete with category A, C and D.
	4.13.1	
C	6.1.1	Freely cross-compete with one another; cross-compete with category B and category D
	3.1.1	
	4.8.1	
	11.2.1	
	11.6.1	
	11.7.1	
D	4.14.3	Cross-compete with category C and B; some cross-competition with category A
E	4.9.1	BNI3 blocks 4.9.1 binding to CTLA4 but not the reverse
	BNI3***	

(*) (**) Available from Biostride.

(***) Available from Pharmingen.

10 As a next step, we endeavored to determine if the antibodies of the invention recognized a linear epitope on CTLA4 under reducing and non-reducing conditions on Western blots. We observed that none of the 4.1.1, 3.1.1, 11.7.1, 11.6.1, or 11.2.1 antibodies of the invention appeared to recognize a reduced form of CTLA4 on Western blot. Accordingly, it appeared likely that
 15 the epitope to which each of these antibodies bound was not a linear epitope but more likely was a conformational epitope the structure of which may have been abrogated under reducing conditions.

Therefore, we sought to determine whether we could learn about residues within the CTLA4 molecule that are important for binding of
 20 antibodies of the invention. One manner that we utilized was to conduct kinetic assessments of off-rates as between human CTLA4 and two highly conserved primate CTLA4 molecules (cynomolgous and marmoset CTLA4). BIAcore

studies demonstrated that the 4.1.1 antibody of the invention bound to human, cynomologous, and marmoset CTLA4 at the same rate. However, with respect to off-rates (affinity), the 4.1.1 antibody had the highest affinity (slowest off-rate) for human, a faster off-rate with cynomologous, and a much faster off-rate for marmoset. The 11.2.1 antibody of the invention, on the other hand, binds to human, cynomologous, and marmoset CTLA4 at the about the same rate and has about the same relative off-rate for each of the three. This information further indicates that the 4.1.1 and 11.2.1 antibodies of the invention bind to different epitopes on CTLA4.

10 To further study the epitope to which the category B and C antibodies of the invention bind, we conducted certain site directed mutagenesis studies. Marmoset CTLA4 possesses two important changes at residues 105 and 106 relative to human CTLA4. Such differences are a leucine to methionine change at residue 105 and a glycine to serine change at residue 106. Accordingly, we mutated cDNA encoding human CTLA4 to encode a mutated CTLA4 having the L105M and G106S changes. The homologue replacement mutant CTLA4 did not effect binding of a B7.2-IgG1 fusion protein. Further, binding with the 11.2.1 antibody of the invention was not effected. However, such molecule was significantly inhibited in its ability to bind with the 4.1.1 antibody of the invention (similar to marmoset). Next, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a S106G change. Such change resulted in restoration of stable binding between the 4.1.1 antibody and the marmoset CTLA4 mutant. In addition, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a M105L change. Such change partially restored binding between the 4.1.1 antibody and the mutant CTLA4.

Each of the category B through D antibodies of the invention appear to possess similar functional properties and appear to have the potential to act as strong anti-CTLA4 therapeutic agents. Further, each of the molecules certain cross-competition in their binding for CTLA4. However, as will be observed from the above discussion, each of the molecules in the different categories appear to bind to separate conformational epitopes on CTLA4.

From the foregoing, it will be appreciated that the epitope information discussed above indicates that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention will likely have certain therapeutic potential in accordance with the present invention. Further, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) will likely have certain additional therapeutic potential in accordance with the present invention. Additionally, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) and that (i) are not reduced in their binding to marmoset CTLA4 (similar to the 11.2.1 antibody) or (ii) are reduced in their binding to marmoset CTLA4 (similar to the 4.1.1 antibody) will likely have certain additional therapeutic potential in accordance with the present invention. Antibodies (or other molecules, as discussed above) that compete with categories A and E may also have certain therapeutic potential.

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

EXAMPLE 1

Generation of Anti-CTLA-4-Antibody Producing Hybridomas

Antibodies of the invention were prepared, selected, and assayed in accordance with the present Example.

Antigen Preparation: Three distinct immunogens were prepared for immunization of the XenoMouseTM mice: (i) a CTLA-4-IgG fusion protein, (ii) a CTLA-4 peptide, and (iii) 300.19 murine lymphoma cells transfected with

a mutant of CTLA-4 (Y201V) that is constitutively expressed on the cell surface.

(i) CTLA-4-IgG1 Fusion Protein:

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Expression Vector Construction:

The cDNA encoding the mature extracellular domain of CTLA-4 was PCR amplified from human thymus cDNA library (Clontech) using primers designed to published sequence (*Eur. J Immunol* 18:1901-1905 (1988)). The fragment was directionally subcloned into pSR5, a Sindbis virus expression plasmid (InVitrogen), between the human oncostatin M signal peptide and human IgG gamma 1 (IgG1) CH1/CH2/CH3 domains. The fusion protein does not contain a hinge domain but contains cysteine 120 in the extracellular domain of CTLA-4 to form a covalent dimer. The resulting vector was called CTLA-4-IgG1/pSR5. The complete CTLA-4-IgG1 cDNA in the vector was sequence confirmed in both strands. The amino acid sequence the CTLA4-Ig protein is shown below. The mature extracellular domain for CD44 was PCR amplified from human lymphocyte library (Clontech) and subcloned into pSinRep5 to generate a control protein with the identical IgG1 tail.

OM-CTLA4-IgG1 Fusion Protein:

MGVLLTORTLLSLVLALLFPSMASMAMHVAQPAVV LASSRG IASFVC
25 EYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICT
GTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGINGTQIY
VIDPEPCPDSLEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPTPIEKTKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
30 VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR
WQGGNVFSCSVMHEALHNHYTQKSLSLSPGK

Underlined: signal peptide

Bold: CTLA4 extracellular domain

5 The cDNAs for mature extracellular domain of CD28 were PCR amplified from human lymphocyte library (Clontech) and then subcloned into pCDM8 (*J. Immunol.* 151: 5261-71 (1993)) to produce a human IgG1 fusion protein containing both thrombin cleavage and hinge regions. Marmoset, Cynomolgous, and Rhesus CTLA4 were cloned from mRNA isolated from
10 PHA stimulated PBMCs using standard techniques of degenerate PCR. Sequencing demonstrated that rhesus and cynomolgous amino acid sequence were identical with three differences from mature human CTLA4 extracellular domain (S13N, I17T and L105M). Marmoset demonstrated ten amino acid differences from the mature human CTLA4 extracellular domain (V21A, V33I,
15 A41T, A51G, 54I, S71F, Q75K, T88M, L105M and G106S). Site directed mutagenesis was used to make single point mutations of all amino acids different in marmoset CTLA4 to map amino acids important for interaction of the antibodies with human CTLA4-IgG. Mutations of human and marmoset CTLA-IgG for epitope mapping were generated by matchmaker site-directed
20 mutagenesis (Promega). The IgG fusion proteins were produced by transient transfection of Cos7 cells and purified using standard Protein A techniques. Mutant CTLA4-IgG proteins were evaluated for binding to antibodies by immunoblotting and using BIAcore analyses.

25 Recombinant Protein Expression/Purification:

Recombinant sindbis virus was generated by electroporating (Gibco) Baby Hamster Kidney cells with SP6 *in vitro* transcribed CTLA-4-IgG1/pSR5 mRNA and DH-26S helper mRNA as described by InVitrogen. Forty eight
30 hours later recombinant virus was harvested and titered for optimal protein expression in Chinese hamster ovary cells (CHO-K1). CHO-K1 cells were cultured in suspension in DMEM/F12 (Gibco) containing 10% heat-inactivated

fetal bovine serum (Gibco), non-essential amino acids (Gibco), 4mM glutamine (Gibco), penicillin/streptomycin (Gibco), 10mM Hepes pH 7.5 (Gibco). To produce CTLA-4-IgG, the CHO-K1 cells were resuspended at 1×10^7 cells/ml in DMEM/F12 and incubated with sindbis virus for one hour at room temperature.

5 Cells were then diluted to 1×10^6 /ml in DMEM/F12 containing 1% fetal bovine serum depleted of bovine IgG using protein A sepharose (Pharmacia), non-essential amino acids, 4mM glutamine, 12.5mM Hepes pH 7.5, and penicillin/streptomycin. Forty eight hours post-infection cells were pelleted and conditioned media was harvested and supplemented with complete protease

10 inhibitor tablets (Boehringer Mannheim), pH adjusted to 7.5, and filtered 0.2 μ (Nalgene). FPLC (Pharmacia) was used to affinity purify the fusion protein using a 5ml protein A HiTrap column (Pharmacia) at a 10ml/min flow rate. The column was washed with 30 bed volumes of PBS and eluted with 0.1M glycine/HCl pH 2.8 at 1ml/min. Fractions (1ml) were immediately

15 neutralized to pH 7.5 with Tris pH 9. The fractions containing CTLA-4-IgG1 were identified by SDS-PAGE and then concentrated using centriplus 50 (Amicon) before applying to sepharose 200 column (Pharmacia) at 1ml/min using PBS as the solvent. Fractions containing CTLA-4-IgG1 were pooled, sterile filtered 0.2 μ (Millipore), aliquoted and frozen at -80°C. CD44-IgG1 was

20 expressed and purified using the same methods. CD28-IgG was purified from conditioned media from transiently transfected Cos7 cells.

Characterization CTLA-4-IgG1:

25 The purified CTLA-4-IgG1 migrated as a single band on SDS-PAGE using colloidal coomassie staining (Novex). Under non-reducing conditions CTLA-4-IgG1 was a dimer (100kDa), that reduced to a 50kDa monomer when treated with 50mM DTT. Amino acid sequencing of the purified CTLA-4-IgG1 in solution confirmed the N-terminus of CTLA-4 (MHVAQPAVVLAS), and

30 that the oncostatin-M signal peptide was cleaved from the mature fusion protein. The CTLA-4-IgG1 bound to immobilized B7.1-IgG in a concentration dependent manner and the binding was blocked by a hamster-anti-human anti-

CTLA-4 antibody (BNI3: PharMingen). The sterile CTLA-4-IgG was endotoxin free and quantitated by OD280 using 1.4 as the extinction coefficient. The yield of purified CTLA-4-IgG ranged between 0.5-3mgs/liter of CHO-K1 cells.

5

(ii) CTLA-4 Peptide:

The following CTLA-4 peptide was prepared as described below:

10 NH₂:MHVAQPAVV LASSRG IASFVCEYASPGKATEVRVTVL RQADSQVT
EVCAATYMMGNELTFLDDSICTGTSSGNQVNLT IQGLRAMDTGLYICK
VELMYPPPYL GIGNGTQIYVIDPEPC-CONH₂

Abbreviations/Materials:

15

NMP, N-Methylpyrrolidinone; TFE, 2,2,2-Trifluoroethanol; DCM, Dichloromethane; Fmoc, Fluorenyl Methoxycarbonyl. All reagents were supplied by Perkin Elmer, with the following exceptions: TFE, Aldrich Chemical, Fmoc-PAL-PEG resin, Perseptive Biosystems. Fmoc-Arg(PMC)-
20 OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-His(Boc)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH and Fmoc-Tyr(tBu)-OH were used for those amino acids requiring side chain protecting groups

25

Peptide Synthesis:

Peptide synthesis was performed on a Perkin-Elmer 431A, retrofitted with feedback monitoring via UV absorbance at 301nm (Perkin-Elmer Model 759A detector). The peptide sequence was assembled on a Fmoc-PAL-PEG
30 resin using conditional double coupling cycles. Forced double couplings were performed at cycles 10,11,18,19,20 and 28 through 33. The resin was washed with a 50% mixture of DCM and TFE at the completion of each acylation cycle,

followed by capping of unreacted amino groups with acetic anhydride in NMP. Resin was removed from the reactor after completing cycle 49 and the remainder continued to completion. Peptide cleavage from the resin was performed using Reagent K (King et al. *International Journal of Protein and Peptide Research* 36:255-266 (1990)) for 6 hours on 415mg of resin affording 186mg crude CTLA-4 peptide.

Peptide Characterization:

10 25mg aliquots of the crude CTLA-4 peptide were dissolved in 5ml 6M Guanidine HCl/100mM K₂PO₃ at pH6.4 and eluted over a Pharmacia Hi Load Superdex 75 16/60 column (16mm x 600mm, 120ml bed volume) with 2M Guanidine.HCl / 100mM K₂PO₃ at pH6.4 at 2 ml / min for 180 minutes collecting 5 ml fractions. The fractions were analyzed by loading 1.7µl of
15 fractions onto a NuPAGE Laemeli gel running with MES running buffer and visualizing via Daichii silver stain protocol. Those fractions exhibiting a molecular weight of 12 KDa, as judged versus molecular weight standards, were pooled together and stored at 4°C. The combined fractions were analyzed by UV and gel electrophoresis. Amino acid sequencing was performed by
20 absorbing a 100 microliter sample in a ProSorb cartridge (absorbed onto a PVDF membrane) and washing to remove the buffer salts. Sequencing was performed on an Applied Biosystems 420. The expected N-terminal sequence (M H V A Q P A V V L A) was observed. Immunoblotting demonstrated that the peptide was recognized by the BNI3 anti-human CTLA-4 (PharMingen).
25 To desalt, an aliquot containing 648µg of material was placed in 3500 Da MWCO dialysis tubing and dialyzed against 0.1% TFA / H₂O at 4°C for 9 days with stirring. The entire contents of the dialysis bag was lyophilized to a powder.

(iii) 300.19 cells transfected with CTLA-4 (Y201V)

The full length CTLA-4 cDNA was PCR amplified from human thymus cDNA library (Stratagene) and subcloned into pIRESneo (Clontech). A mutation of CTLA-4 that results in constitutive cell surface expression was introduced using MatchMaker Mutagenesis System (Promega). Mutation of tyrosine, Y201 to valine inhibits binding of the adaptin protein AP50 that is responsible for the rapid internalization of CTLA-4 (Chuang et al. *J. Immunol.* 159:144-151 (1997)). Mycoplasma-free 300.19 murine lymphoma cells were cultured in RPMI-1640 containing 10% fetal calf serum, non-essential amino acids, penicillin/streptomycin, 2mM glutamine, 12.5mM Hepes pH 7.5, and 25uM beta-mercaptoethanol. Cells were electroporated (3×10^6 /0.4ml serum free RPMI) in a 1ml chamber with 20ug CTLA-4-Y201V/pIRESneo using 200V/1180uF (Gibco CellPorator). Cells were rested for 10 minutes and then 8mls of prewarmed complete RPMI media. At 48 hours cells were diluted to 0.5×10^6 /ml in complete RPMI media containing 1mg/ml G418 (Gibco). Resistant cells were expanded and shown to express CTLA-4 on the cell surface using the BNI3 antibody conjugated with phycoerythrin (PharMingen). High level expressing cells were isolated by sterile sorting.

20

Immunization and hybridoma generation: XenoMouse mice (8 to 10 weeks old) were immunized (i) subcutaneously at the base of tails with 1×10^7 300.19 cells that were transfected to express CTLA-4 as described above, resuspended in phosphate buffered saline (PBS) with complete Freund's adjuvant, or (ii) subcutaneously at the base of tail with (a) 10 μ g the CTLA-4 fusion protein or (b) 10 μ g CTLA-4 peptide, emulsified with complete Freund's adjuvant. In each case, the dose was repeated three or four times in incomplete Freund's adjuvant. Four days before fusion, the mice received a final injection of the immunogen or cells in PBS. Spleen and/or lymph node lymphocytes from immunized mice were fused with the [murine non-secretory myeloma P3 cell line] and were subjected to HAT selection as previously described (Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and

30

procedures." *Methods Enzymol.* 73:3-46 (1981)). A large panel of hybridomas all secreting CTLA-4 specific human IgG₂κ or IgG₄κ (as detected below) antibodies were recovered.

5 **ELISA assay:** ELISA for determination of antigen-specific antibodies in mouse serum and in hybridoma supernatants was carried out as described (Coligan et al., Unit 2.1, "Enzyme-linked immunosorbent assays," in *Current protocols in immunology* (1994)) using CTLA-4-Ig fusion protein to capture the antibodies. For animals that are immunized with the CTLA-4-Ig fusion protein,
10 we additionally screen for non-specific reactivity against the human Ig portion of the fusion protein. This is accomplished using ELISA plates coated with human IgG1 as a negative control for specificity.

In a preferred ELISA assay, the following techniques are used:

ELISA plates are coated with 100 μl/well of the antigen in plate coating
15 buffer (0.1 M Carbonate Buffer, pH 9.6 and NaHCO₃ (MW 84) 8.4g/L). Plates are then incubated at 4°C overnight. After incubation, coating buffer is removed and the plate is blocked with 200 μl/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 hour. Alternatively, the plates are stored in refrigerator with
20 blocking buffer and plate sealers. Blocking buffer is removed and 50 μl/well of hybridoma supernatant, serum or other hybridoma supernatant (positive control) and HAT media or blocking buffer (negative control) is added. The plates are incubated at room temperature for 2 hours. After incubation, the plate is washed with washing buffer (1x PBS). The detecting antibody (i.e., mouse anti-
25 human IgG2-HRP (SB, #9070-05) for IgG2 antibodies or mouse anti-human IgG4-HRP (SB #9200-05) for IgG4 antibodies) is added at 100μl/well (mouse anti-human IgG2-HRP @ 1:2000 or mouse anti-human IgG4-HRP @ 1:1000 (each diluted in blocking buffer)). The plates are incubated at room temperature for 1 hour and then washed with washing buffer. Thereafter, 100 μl/well of
30 freshly prepared developing solution (10 ml Substrate buffer, 5 mg OPD (o-phenylenediamine, Sigma Cat No. P-7288), and 10 μl 30% H₂O₂ (Sigma)) is added to the wells. The plates are allowed to develop 10-20 minutes, until

negative control wells barely start to show color. Thereafter, 100 μ l/well of stop solution (2 M H_2SO_4) is added and the plates are read on an ELISA plate reader at wavelength 490 nm.

5 **Determination of affinity constants of fully human Mabs by BIAcore:**

Affinity measurement of purified human monoclonal antibodies, Fab fragments, or hybridoma supernatants by plasmon resonance was carried out using the BIAcore 2000 instrument, using general procedures outlined by the manufacturers.

10 Kinetic analysis of the antibodies was carried out using antigens immobilized onto the sensor surface at a low density. Three surfaces of the BIAcore sensorchip were immobilized with the CTLA-4-Ig fusion protein at a density ranging from approximately 390-900 using CTLA-4-Ig fusion protein at 20 or 50 μ g/ml in 10 mM sodium acetate at pH 5.0 using the amine coupling kit
15 supplied by the manufacturer (BIAcore, Inc.). The fourth surface of the BIAcore sensorchip was immobilized with IgG1 (900 RU) and was used as a negative control surface for non-specific binding. Kinetic analysis was performed at a flow rate of 25 or 50 microliters per minute and dissociation (k_{off} or k_{off}) and association (k_a or k_{on}) rates were determined using the software
20 provided by the manufacturer (BIA evaluation 3.0) that allows for global fitting calculations.

EXAMPLE 2

Affinity Measurement of Anti-CTLA-4-Antibodies

25

In the following Table, affinity measurements for certain of the antibodies selected in this manner are provided:

TABLE I

Hybridoma	Solid Phase (by BIAcore)				
	On-rates K_a ($M^{-1}S^{-1} \times 10^6$)	Off-rates K_d ($S^{-1} \times 10^{-4}$)	Association Constant $KA (1/M) =$ $k_a/k_d \times 10^{10}$	Dissociation Constant $KD(M) =$ $K_d/k_a \times 10^{-10}$	Surface Density [RU]
Moab01	0.68	1.01	0.67	1.48	878.7
	0.70	4.66	0.15	6.68	504.5
	0.77	6.49	0.19	8.41	457.2
	0.60	3.08	0.20	5.11	397.8
4.1.1	1.85	0.72	2.58	0.39	878.7
	1.88	1.21	1.55	0.64	504.5
	1.73	1.54	1.13	0.88	457.2
	1.86	1.47	1.26	0.79	397.8
4.8.1	0.32	0.07	4.46	0.22	878.7
	0.31	0.23	1.33	0.75	504.5
	0.28	0.06	4.82	0.21	397.8
4.14.3	2.81	3.04	0.92	1.08	878.7
	2.88	3.97	0.73	1.38	504.5
	2.84	6.66	0.43	2.35	457.2
	3.17	5.03	0.63	1.58	397.8
6.1.1	0.43	0.35	1.21	0.83	878.7
	0.46	0.90	0.51	1.98	504.5
	0.31	0.51	0.61	1.63	457.2
	0.45	0.79	0.57	1.76	397.8
3.1.1	1.04	0.96	1.07	0.93	878.7
	0.95	1.72	0.55	1.82	504.5
	0.73	1.65	0.44	2.27	457.2
	0.91	2.07	0.44	2.28	397.8
4.9.1	1.55	13.80	0.11	8.94	878.7
	1.43	19.00	0.08	13.20	504.5
	1.35	20.50	0.07	15.20	397.8
4.10.2	1.00	2.53	0.39	2.54	878.7
	0.94	4.30	0.22	4.55	504.5
	0.70	5.05	0.14	7.21	457.2
	1.00	5.24	0.19	5.25	397.8
2.1.3	1.24	9.59	0.13	7.72	878.7
	1.17	13.10	0.09	11.20	504.5
	1.11	13.00	0.09	11.70	397.8
4.13.1	1.22	5.83	0.21	4.78	878.7
	1.29	6.65	0.19	5.17	504.5
	1.23	7.25	0.17	5.88	397.8

As will be observed, antibodies prepared in accordance with the invention possess high affinities and binding constants.

5

EXAMPLE 3

Structures of Anti-CTLA-4-Antibodies Prepared in Accordance with the Invention

In the following discussion, structural information related to antibodies
10 prepared in accordance with the invention is provided.

In order to analyze structures of antibodies produced in accordance with the invention, we cloned genes encoding the heavy and light chain fragments out of the particular hybridoma. Gene cloning and sequencing was accomplished as follows:

15 Poly(A)⁺ mRNA was isolated from approximately 2 X 10⁵ hybridoma cells derived from immunized XenoMouse mice using a Fast-Track kit (Invitrogen). The generation of random primed cDNA was followed by PCR. Human V_H or human V_K family specific variable region primers (Marks et al., "Oligonucleotide primers for polymerase chain reaction amplification of human
20 immunoglobulin variable genes and design of family-specific oligonucleotide probes." *Eur. J. Immunol.* 21:985-991 (1991)) or a universal human V_H primer, MG-30 (CAGGTGCAGCTGGAGCAGTCIGG) was used in conjunction with primers specific for the human C_γ2 constant region (MG-40d; 5'-GCTGAGGGAGTAGAGTCCTGAGGA-3') or C_κ constant region (hκP2;
25 as previously described in Green et al., 1994). Sequences of human Mabs-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly(A⁺) RNA using the primers described above. PCR products were also cloned into pCRII using a TA cloning kit (Invitrogen) and both strands were sequenced using
30 Prism dye-terminator sequencing kits and an ABI 377 sequencing machine. All sequences were analyzed by alignments to the "V BASE sequence directory"

(Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK) using MacVector and Geneworks software programs.

Further, each of the antibodies 4.1.1, 4.8.1, 11.2.1, and 6.1.1 were subjected to full length DNA sequences. For such sequencing, Poly(A)⁺ mRNA was isolated from approximately 4 X 10⁶ hybridoma cells using mRNA Direct kit (Dynal). The mRNA was reverse transcribed using oligo-dT(18) and the Advantage RT/PCR kit (Clonotech). The Variable region database (V Base) was used to design amplification primers beginning at the ATG start site of the heavy chain DP50 gene (5'-
 10 TATCTAAGCTTCTAGACTCGACCGCCACCATGGAGTTTGGGCTGAGC
 TG-3') and to the stop codon of the IgG2 constant region (5'-
 TTCTCTGATCAGAATTCCTATCATTTACCCGGAGACAGGGAGAGCT-
 3'). An optimal Kozak sequence (ACCGCCACC) was added 5' to the ATG
 start site. The same method was used to design a primer to the ATG start site of
 15 the kappa chain A27 gene (5'-
 TCTTCAAGCTTGCCCGGGCCCGCCACCATGGAAACCCAGCGCAG
 -3') and the stop codon of the kappa constant region (5'-
 TTCTTTGATCAGAATTCTCACTAACAACCTCTCCCCTGTTGAAGC-3').
 The 012 cDNA was cloned by using a primer to the ATG start site (5'-
 20 TCTTCAAGCTTGCCCGGGCCCGCCACCATGGACATGAGGGTCCCCGC
 T-3) and the kappa constant region stop codon primer above. The heavy chain
 cDNAs were also cloned as genomic constructs by site directed mutagenesis to
 add an NheI site at the end of the variable J domain and subcloning an NheI-
 fragment containing the genomic IgG2 CH1/Hinge/CH2/CH3 regions. The
 25 point mutation to generate NheI site does not alter the amino acid sequence
 from germline. The primer pairs were used to amplify the cDNAs using
 Advantage High Fidelity PCR Kit (Clonotech). Sequence of the PCR was
 obtained by direct sequencing using dye-terminator sequencing kits and an ABI
 sequencing machine. The PCR product was cloned into pEE glutamine
 30 synthetase mammalian expression vectors (Lonza) and three clones were
 sequenced to confirm somatic mutations. For each clone, the sequence was
 verified on both strands in at least three reactions. An aglycosylated 4.1.1

antibody was generated by site directed mutagenesis of N294Q in the CH2 domain. Recombinant antibodies were produced by transient transfection of Cos7 cells in IgG depleted FCS and purified using standard Protein A sepharose techniques. Stable transfectants were generated by electroporation of murine
5 NSO cells and selection in glutamine free media. Recombinant 4.1.1 with or without glycosylation exhibited identical specificity and affinity for CTLA4 in the *in vitro* ELISA and BIAcore assays.

Gene Utilization Analysis

10

The following Table sets forth the gene utilization evidenced by selected hybridoma clones of antibodies in accordance with the invention:

TABLE II
Heavy and Light Chain Gene Utilization

Clone	Heavy Chain				Kappa Light Chain	
	VH	D	JH		VK	JK
4.1.1	DP-50	DIR4 or DIR3	JH4		A27	JK1
4.8.1	DP-50	7-27	JH4		A27	JK4
4.14.3	DP-50	7-27	JH4		A27	JK3
6.1.1	DP-50	DIR5 or DIR5rc	JH4		A27	JK3
3.1.1	DP-50	3-3	JH6		012	JK3
4.10.2	DP-50	7-27	JH4		A27	JK3
2.1.3	DP-65	1-26	JH6		A10/A26	JK4
4.13.1	DP-50	7-27	JH4		A27	JK3
11.2.1	DP-50	D1-26	JH6		012	JK3
11.6.1	DP-50	D2-2 or D4	JH6		012	JK3
11.7.1	DP-50	D3-22 or D21-9	JH4		012	JK3
12.3.1.1	DP-50	D3-3 or DXP4	JH6		A17	JK1
12.9.1.1	DP-50	D6-19	JH4		A3/A19	JK4
4.9.1	DP-47	5-24 and/or 6-19	JH4		L5	JK1

5

As will be observed, antibodies in accordance with the present invention were generated with a strong bias towards the utilization of the DP-50 heavy chain variable region. The DP-50 gene is also referred to as a V_H 3-33 family gene. Only one antibody that was selected on the basis of CTLA-4 binding and preliminary *in vitro* functional assays showed a heavy chain gene utilization other than DP-50. That clone, 2.1.3, utilizes a DP-65 heavy chain variable region and is an IgG4 isotype. The DP-65 gene is also referred to as a V_H 4-31 family gene. On the other hand, the clone, 4.9.1, which possesses a DP-47 heavy chain variable region binds to CTLA-4 but does not inhibit binding to B7-1 or B7-2. In XenoMouse mice, there are more than 30 distinct functional

15

heavy chain variable genes with which to generate antibodies. Bias, therefore, is indicative of a preferred binding motif of the antibody-antigen interaction with respect to the combined properties of binding to the antigen and functional activity.

5

Mutation Analysis

As will be appreciated, gene utilization analysis provides only a limited overview of antibody structure. As the B-cells in XenoMouse animals stochastically generate V-D-J heavy or V-J kappa light chain transcripts, there are a number of secondary processes that occur, including, without limitation, somatic hypermutation, n-additions, and CDR3 extensions. See, for example, Mendez et al. *Nature Genetics* 15:146-156 (1997) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996. Accordingly, to further examine antibody structure predicted amino acid sequences of the antibodies were generated from the cDNAs obtained from the clones. In addition, N-terminal amino acid sequences were obtained through protein sequencing.

Figure 1 provides nucleotide and predicted amino acid sequences of the heavy and kappa light chains of the clones 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M). In Figures 1A, 1B, and 1D, extended sequences of the antibodies 4.1.1, 4.8.1, and 6.1.1 were obtained by full length cloning of the cDNAs as described above. In such Figures, the signal peptide sequence (or the bases encoding the same) are indicated in bold and sequences utilized for the 5' PCR reaction are underlined.

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The

30

Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "Os".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17 amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the

positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains:

4.1.1:

full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c));

full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e));

4.1.1 light chain (cDNA 22(f) and amino acid 22(g));

4.8.1:

full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i));

4.8.1 light chain (cDNA 22(j) and amino acid 22(k));

6.1.1:

full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m));

6.1.1 light chain (cDNA 22(n) and amino acid 22(o));

11.2.1:

full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)); and

11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

Signal peptide sequences are shown in bold and large text. The open reading frames in the full length 4.1.1 genomic DNA sequence (Fig. 22(b)) are underlined. And, the mutations introduced to make the aglycosylated 4.1.1 heavy chain and the resulting change (N294Q) are shown in double underline and bold text (cDNA (Fig. 22(b)) and amino acid (Fig. 22(c))).

EXAMPLE 4

Analysis of Heavy and Light Chain Amino Acid Substitutions

10 In Figure 2, which provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence, an interesting pattern emerges. In addition to the fact of the bias for heavy chain DP-50 in the majority of the clones, there is relatively limited hypermutation in the antibodies relative to the germline DP-50 gene. For example, clones 3.1.1 and 11.2.1 have no mutations. Moreover, the mutations in the other clones are generally conservative changes, involving substitutions of amino acids with similar properties to the amino acids in the germline. Mutations within many of the CDR1 and CRD2 sequences are particularly conservative in nature. Three of the heavy chains represented in Figure 2, 4.10.2, 4.13.1, and 4.14.3, are clearly derived from a single recombination event (i.e., derive from an identical germinal center) and are nearly identical in sequence. If these three are considered as a single sequence, then, among the 10 different antibodies containing the DP50 heavy chain, in CDR1 and CDR2 there are 3 positions in which a nonpolar residue is replaced by another nonpolar residue, 12 in which a polar uncharged residue is replaced by another polar uncharged residue, and 1 in which a polar charged residue is replaced by another polar charged residue. Further, there are two positions in which two residues which are very similar structurally, glycine and alanine, are substituted for one another. The only mutations not strictly conservative involve 3 substitutions of a polar charged residue for a polar uncharged residue and one substitution of a nonpolar residue for a polar residue.

The light chains of these antibodies are derived from 5 different Vk genes. The A27 gene is the most heavily represented and is the source of 6 different light chains. Comparison of these 6 sequences reveals two noteworthy features. First, in three of them, 4.8.1, 4.14.3, and 6.1.1, contain deletions of one or two residues in CDR1, a rare event. Second, there is a strong prejudice against the germline serine at position six in CDR3 in that the serine has been replaced in every sequence. This suggests that a serine at this position is incompatible with CTLA4 binding.

It will be appreciated that many of the above-identified amino acid substitutions exist in close proximity to or within a CDR. Such substitutions would appear to bear some effect upon the binding of the antibody to the CTLA-4 molecule. Further, such substitutions could have significant effect upon the affinity of the antibodies.

EXAMPLE 5

N-Terminal Amino Acid Sequence Analysis of Antibodies in Accordance with the Invention

In order to further verify the composition and structure of the antibodies in accordance with the invention identified above, we sequenced certain of the antibodies using a Perkin-Elmer sequencer. Both heavy and kappa light chains of the antibodies were isolated and purified through use of preparative gel electrophoresis and electroblotting techniques and thereafter directly sequenced as described in Example 6. A majority of the heavy the heavy chain sequences were blocked on their amino terminus. Therefore, such antibodies were first treated with pyroglutamate aminopeptidase and thereafter sequenced.

The results from this experiment are shown in Figure 9. Figure 9 also provides the molecular weight of the heavy and light chains as determined by mass spectroscopy (MALDI).

EXAMPLE 6**Additional Characterization of Antibodies in Accordance with the
Invention**

5 Figure 10 provides certain additional characterizing information about certain of the antibodies in accordance with the invention. In the Figure, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. The following data is provided: Concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, FACS, mass spectroscopy (MALDI),
10 and light chain N-terminal sequences.

Generally, the data was generated as follows:

Materials and Methods

15 Protein concentration was determined at 280 nm from a UV scan (200-350 nm), where 1.58 absorbance units at 280 nm equaled 1mg/ml.

SDS-PAGE was performed using the Novex NuPAGE electrophoresis system with a 10% NuPAGE gel and MES running buffer. Samples were
20 prepared by diluting 3:1 with 4x NuPAGE sample buffer (+/-) beta-mercaptoethanol, heated and ~ 5 ug of protein was loaded onto the gel. The gel was then stained with Brilliant Blue R staining solution (Sigma cat.# B-6529) and molecular size estimates were made by comparing stained bands to "Perfect Protein Markers" (Novagen cat# 69149-3).

25 For N-terminal sequencing, samples were run as above on NuPAGE gels, transferred to Pro Blot immobilization membrane (Applied Biosystems) then stained with Coomassie Blue R-250. The stained protein bands were excised and subjected to sequence analysis by automated Edman degradation on
30 an Applied Biosystems 494 Procise HT Sequencer.

Isoelectric focusing (IEF) was performed using Pharmacia IEF 3-9 pHast gels (cat# 17-0543-01). Samples were diluted in 10% glycerol to ~0.8 mg/ml and 1ul was loaded onto gel and then silver stained. The pI estimates were made by comparing stained bands to broad range (pH3-10) IEF standards
5 (Pharmacia cat # 17-0471-01)

Size exclusion chromatography (SEC) was carried in phosphate buffered saline (PBS) on the Pharmacia SMART system using the Superdex 75 PC 3.2/30 column. Molecular size estimates were made by comparing peak
10 retention time to the retention times of gel

For FACS studies, human peripheral T cells were prepared and stimulated for 48 hours. T cells were washed once, resuspended in FACS buffer at 1×10^6 cells/100 ul and stained for CD3 surface expression with 10 ul
15 of anti-CD3-FITC (Immunotech, Marseille, France) for 30 minutes at room temperature. Cells were washed twice, then fixed, permeabilized (Fix and Perm, Caltag), and stained for intracellular CTLA-4 expression with 10 ul anti-CD152-PE (Pharmingen). Flow cytometry was performed using a Becton Dickinson FACSort. Quadrants were set by analysis of relevant isotype control
20 antibodies (Caltag).

As was discussed above, anti-CTLA-4 antibodies have been demonstrated to possess certain powerful immune modulation activities. The
25 following experiments were carried out in order to determine if antibodies in accordance with the present invention possessed such activities. In general, the experiments were designed to assess ability of the antibodies to inhibit the interaction between CTLA-4 and B7 molecules, be selective as between CTLA-4 and B7 molecules and CD28, and promote T cell cytokine production,
30 including, but not limited to IL-2 and/or IFN- γ expression. Further, examination of cross-reactivity of antibodies of the invention with certain

human tissues and CTLA-4 molecules in other species (e.g., mouse and primate) was undertaken.

EXAMPLE 7

5 Competition ELISA: Inhibition of CTLA-4/B7-1 or B7-2 Interaction by Antibodies in Accordance with the Invention

 An *in vitro* assay was conducted to determine if antibodies in accordance with the present invention were capable of inhibiting the binding of
10 CTLA-4 with either B7-1 or B7-2. As will be appreciated, antibodies of the invention that are capable of inhibiting the binding of CTLA-4 with B7 molecules would be expected to be candidates for immune regulation through the CTLA-4 pathway. In the assay, the following materials and methods were utilized:

15

Materials and Methods

 3 nM B7.1-Ig(G1) or B7.2-Ig(G1) (Repligen, Inc. Needham, MA) in Dulbecco's PBS was coated on 96-well MaxiSorp plates (Nunc, Denmark,
20 #439454) and incubated at 4°C overnight. On day 2, B7-Ig was removed and plates were blocked with 1% BSA plus 0.05% Tween-20 in D-PBS for two hours. Plates were washed 3X with wash buffer (0.05% Tween-20 in D-PBS). Antibody at appropriate test concentrations and CTLA-4-Ig(G4) (0.3 nM final conc.) (Repligen, Inc. Needham, MA) were pre-mixed for 15 minutes and then
25 added to the B7-Ig coated plate (60 ul total volume) and incubated at RT for 1.5 hours. Plates were washed 3X and 50 µl of a 1 to 1000 dilution of HRP-labeled mouse anti-human IgG4 antibody (Zymed, San Francisco, CA, #05-3820) was added and incubated at RT for 1 hour. Plates were washed 3X and 50 µl TMB Microwell peroxidase substrate (Kirkegaard & Perry, Gaithersburg, MD, #50-
30 76-04) was added and incubated at RT for 20 minutes, and then 50 µl 1N H₂SO₄ was added to the plate. Plates were read at 450 nm using a Molecular Devices plate reader (Sunnyvale, CA). All samples were tested in duplicate. Maximal

signal was defined as CTLA-4-Ig binding in the absence of test antibody. Non-specific binding was defined as absorbance in the absence of CTLA-4-Ig and test antibody.

5 The results from the assay are provided in Table IIIA and IIIB. In Table IIIA, results are shown for a variety of antibodies in accordance with the invention. In Table IIIB, results are shown comparing the 4.1.1 antibody of the invention with the 11.2.1 antibody of the invention from a separate experiment.

10

TABLE IIIA

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT3.1.1	IgG2	0.45 ± 0.07 (n=3)	0.63 ± 0.10 (n=2)
CT4.1.1	IgG2	0.38 ± 0.06 (n=5)	0.50 ± 0.05 (n=2)
CT4.8.1	IgG2	0.57 ± 0.03 (n=3)	0.17 ± 0.28 (n=2)
CT4.9.1	IgG2	Non-competitive (n=3)	non-competitive (n=2)
CT4.10.2	IgG2	1.50 ± 0.37 (n=3)	3.39 ± 0.31 (n=2)
CT4.13.1	IgG2	0.49 ± 0.05 (n=3)	0.98 ± 0.11 (n=2)
CT4.14.3	IgG2	0.69 ± 0.11 (n=3)	1.04 ± 0.15 (n=2)
CT6.1.1	IgG2	0.39 ± 0.06 (n=3)	0.67 ± 0.07 (n=2)

15

TABLE IIIB

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT4.1.1	IgG2	0.55 ± 0.08 (n=4)	0.87 ± 0.14 (n=2)
CT11.2.1	IgG2	0.56 ± 0.05 (n=4)	0.81 ± 0.24 (n=2)

EXAMPLE 8**Selectivity Ratios of Antibodies of the Invention with Respect to CTLA-4
Versus Either CD28 or B7-2**

5 Another *in vitro* assay was conducted to determine the selectivity of antibodies of the invention with respect to CTLA-4 versus either CD28 or B7-2. The following materials and methods were utilized in connection with the experiments:

10 **CTLA-4 Selectivity ELISA: Materials and Methods**

A 96-well FluroNUNC plate (Nunc Cat No.475515) was platecoated with four antigens: CTLA-4/Ig, CD44/Ig, CD28/Ig, and B7.2/Ig (antigens generated in-house). The antigens were platecoated overnight at +4°C at 1ug/ml
15 100ul/well in 0.1M sodium bicarbonate buffer, pH 9.6. The plate was then washed with PBST (PBS + 0.1% Tween-20) three times using a NUNC plate washer. The plate was blocked with PBST+0.5%BSA at 150 ul/well. The plate was incubated at RT for 1 hour then washed with PBST three times. Next the anti-CTLA-4 antibodies of the invention were diluted in block at 1 µg/ml and
20 were added to the plate. The plate was incubated at RT for 1 hour then washed with PBST three times. The wells that contained the antibodies of the invention were then treated with 100 µl/well anti-human IgG2-HRP (Southern Biotech Cat No.9070-05) at a 1:4000 dilution in block. Also, one row was treated with anti-human IgG (Jackson Cat No. 209-035-088) to normalize for platecoating.
25 This antibody was diluted to 1:5000 in block and added at 100 ul/well. Also, one row was treated with anti-human CTLA-4-HRP (Pharmingen Cat No. 345815/Custom HRP conjugated) as a positive control. This antibody was used at 0.05 ug/ml diluted in block. The plate was incubated at RT for 1 hour then washed with PBST three times. LBA chemiluminescent substrate (Pierce) was
30 added at 100 µl/well and the plate was incubated on a plateshaker for 5 min. The plate was then read using a lumi-imager for a 2 min. exposure.

IGEN CTLA-4-Ig Selectivity Binding Assay: Materials and Methods

M-450 Dynabeads (Dynal A.S, Oslo, Norway #140.02) were washed 3X with Na phosphate buffer, pH 7.4 and resuspended in Na phosphate buffer. 1.0 μ g CTLA-4-Ig(G1), 1.0 μ g CD28-Ig(G1) or 1.0 to 3.0 μ g B7.2-Ig(G1) (Repligen, Inc. Needham, MA) were added to 100 μ l of beads and incubated overnight on a rotator at 4°C. On day 2 the beads were washed 3X in 1% BSA plus 0.05% Tween-20 in Dulbecco's PBS and blocked for 30 minutes. Beads were diluted 1 to 10 with blocking buffer and 25 μ l of the coated beads were added to 12x75 mm polypropylene tubes. All samples were tested in duplicate. 50 μ l test antibody (1 μ g/ml final concentration) or blocking buffer was added to the tubes and incubated for 30 minutes on the Origen 1.5 Analyzer carousel (IGEN International, Inc., Gaithersburg, MD) at RT, vortexing at 100 rpm. 25 μ l of ruthenylated murine anti-human IgG1, IgG2 or IgG4 (Zymed, Inc. San Francisco, CA #05-3300, 05-3500 and 05-3800) (final concentration of 3 μ g/ml in 100 μ l total volume) was added to the tubes. Tubes were incubated for 30 minutes at RT on the carousel vortexing at 100 rpm. 200 μ l of Origen assay buffer (IGEN International, Inc., Gaithersburg, MD #402-050-03) per tube was added and briefly vortexed and then the tubes were counted in the Origen Analyzer and ECL (electrochemiluminescence) units were determined for each tube. Normalization factors were determined to correct for differences in binding of fusion proteins to Dynabeads, and ECL units were corrected for non-specific binding before calculating selectivity ratios.

The results from the assays are provided in Tables IVA and IVB.

TABLE IVA

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/CD44 ELISA	CTLA4/CD28 IGEN	CTLA4/B7.2 IGEN
3.1.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=1) 195:1 (n=1)
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 485:1 (n=1)	>500:1 (n=3)	>500:1 (n=1) 261:1 (n=1)	>500:1 (n=1) 107:1 (n=1)
4.8.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 190:1 (n=1)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)
4.9.1	IgG2	>500:1 (n=2) 244:1 (n=1)	>500:1 (n=2) 33:1 (n=1)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.10.2	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.13.1	IgG2	>500:1 (n=2) 46:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1) 329:1 (n=1)	>500:1 (n=2)
4.14.3	IgG2	>500:1 (n=2) 80:1 (n=1)	>500:1 (n=2) 10:1 (n=1)	>500:1 (n=2) 126:1 (n=1)	>413:1 (n=1)	>234:1 (n=1)
6.1.1	IgG2	>500:1 (n=2) 52:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)

5

TABLE IVB

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/hIgG ELISA
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=3)
11.2.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)

10

EXAMPLE 9**Human T-Cell Signal Model**

15

In order to further define the activity of antibodies in accordance with the invention to act as immune regulators, we developed certain T-cell assays in order to quantify the enhancement of T-cell IL-2 production upon blockade of

CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:

Materials and Methods

5

Freshly isolated human T cells were prepared by using Histopaque (Sigma, St. Louis, MO #A-70543) and T-kwik (Lympho-Kwik, One Lambda, Canoga Park, CA, #LK-50-T), and stimulated with PHA (1 μ g/ml) (Purified Phytohemagglutinin, Murex Diagnostics Ltd. Dartford, England, #HA 16) in medium (RPMI 1640 containing L-glutamine, MEM non-essential amino acids, penicillin, streptomycin, 25 mM Hepes and 10% FBS) at a concentration of 1×10^6 cells/ml and incubated at 37°C for 2 days. The cells were washed and diluted in medium to 2×10^6 cells/ml. Raji cells (Burkitt lymphoma, Human ATCC No.: CCL 86 Class II American Type Culture Collection Rockville, MD) were treated with mitomycin C (Sigma St. Louis, MO, # M-4287) (25 μ g/ml) for one hour at 37°C. The Raji cells were washed 4X in PBS and resuspended at 2×10^6 cells/ml. Human T cell blasts (5×10^5 /ml), Raji cells (5×10^5 /ml) and anti-CTLA-4 antibodies or an isotyped-matched control antibody at various concentrations were added to 96-well microtiter plates and the plates were incubated at 37°C for 72 hours. Total volume per well was 200 μ l. Seventy-two hours post stimulation, the plates were spun down and supernatant removed and frozen for later determination of IL-2 (Quantikine IL-2 ELISA kit, R&D Systems, Minneapolis, MN, #D2050) and IFN- γ (Quantikine IFN-g ELISA kit, R&D Systems). Cytokine enhancement was defined as the difference between cytokine levels in cultures containing an anti-CTLA-4 blocking mAb versus an isotype-matched control antibody. For flow cytometry experiments, Raji cells were washed 1x with FACS buffer (PBS containing 2% heat inactivated FCS, 0.025% sodium azide). Cell pellets were resuspended in FACS buffer at 1×10^6 cells/100 μ l and incubated with 10 μ l of anti-CD80-PE (Becton Dickinson, San Jose, CA) or anti-CD86-PE (Pharmingen, San Diego, CA) for 30 minutes at room temperature. Cells were washed twice and resuspended in 1 ml FACS buffer. Flow cytometry was performed using a Becton Dickinson FACSsort.

30

Histogram markers were set by analysis of relevant isotype control antibodies (Caltag, Burlingame, CA).

In general, we have developed an assay that can be used for rapid
5 determination of T-cell IL-2 upregulation. As will be appreciated, stimulation of T cells is B7 and CD28 dependent. Further, washed T blasts do not make detectable IL-2 and Raji cells do not make detectable IL-2 even when stimulated with LPS or PWM. However, in combination, the T blasts co-cultured with Raji cells can model B7, CTLA-4, and CD28 signaling events and
10 the effects of antibodies thereon can be assessed.

Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs using flow cytometry (FACs) as described in Example 6.

Figure 12 shows the concentration dependent enhancement of IL-2
15 production in the T cell blast/Raji assay induced by CTLA-4 blocking antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention).

Figure 13 shows the concentration dependent enhancement of IFN- γ production in the T cell blast/Raji assay induced by CTLA-4 blocking
20 antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) (same donor T cells).

Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay. It is interesting to consider that the mAb, CT4.9.1, binds to CTLA4 but
25 does not block B7 binding. Thus, simply binding to CTLA-4 is insufficient by itself to provide a functional antibody of the invention.

Figure 15 shows the mean enhancement of IFN- γ production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay.

30 Figure 19 shows a comparison between the 4.1.1 and 11.2.1 antibodies of the invention at a concentration of 30 μ g/ml in the 72 hour T cell blast/Raji

assay as described in this Example 9 and the Superantigen assay described in Example 10.

Figure 20 shows the concentration dependent enhancement of IL-2 production in the T cell blast/Raji assay induced by the 4.1.1 and 11.2.1 CTLA4 antibodies of the invention.

The following Table IVc provides information related to mean enhancement and range of enhancement of cytokine response in the Raji and SEA assays of the invention. Each of the experiments included in the results are based on antibody at a dose of 30 μ g/ml and measured at 72 hours. Numbers of donors used in the experiments as well as responses are shown.

TABLE IVc

Assay	mAb	Cytokine	Mean Enhancement pg/ml	SEM	Range Enhancement pg/ml	n	Donor Response
T cell blast/Raji	4.1.1	IL-2	3329	408	0 to 8861	42	19 of 21
T cell blast/Raji	4.1.1	IFN- γ	3630	980	600 to 13939	17	13 of 13
T cell blast/Raji	11.2.1	IL-2	3509	488	369 to 6424	18	14 of 14
SEA (PBMC)	4.1.1	IL-2	2800	312	330 to 6699	42	17 of 17
SEA (PBMC)	11.2.1	IL-2	2438	366	147 to 8360	25	15 of 15
SEA (Whole Blood)	4.1.1	IL-2	6089	665	-168 to 18417	46	15 of 17
SEA (Whole Blood)	11.2.1	IL-2	6935	700	-111 to 11803	25	12 of 14

EXAMPLE 10

Human T-Cell Signal Model

We developed a second cellular assay in order to quantify the enhancement of T-cell IL-2 upregulation upon blockade of CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:

Materials and Methods

Human PBMC were prepared using Accuspin. Microtiter plates were precoated with an anti-CD3 antibody (leu4, Becton Dickinson) (60 ng/ml) and incubated for 2 hours at 37⁰C. hPBMC were added to the wells at 200,000 cells per well. Staphylococcus enterotoxin A (SEA) (Sigma) was added to the wells at 100 ng/ml. Antibodies were added to the wells, usually at 30 µg/ml. Cells were then stimulated for 48, 72 or 96 hours. Plates were centrifuged at the desired time-point and supernatants were removed from the wells. Thereafter, supernatants were checked for IL-2 production using ELISA (R&D Systems).

Results from these experiments are shown in Figures 16, 17, and 21. In Figure 16, induction of IL-2 production in hPBMC from 5 donors was measured 72 hours after stimulation. In Figure 17, results are shown from measurement of whole blood, analyzing the difference in induction of IL-2 production in the blood of 3 donors as measured at 72 and 96 hours after stimulation.

In Figure 21, the enhancement of IL-2 production in whole blood of 2 donors as measured at 72 hours after stimulation.

EXAMPLE 11

Tumor Animal Model

We have established an animal tumor model for the *in vivo* analysis of anti-murine-CTLA-4 antibodies in inhibiting tumor growth. In the model, a murine fibrosarcoma tumor is grown and the animals are treated with anti-murine-CTLA-4 antibodies. The materials and methods for establishment of the model are provided below:

Materials and Methods

Female A/J mice (6-8 weeks old) were injected subcutaneously on the dorsal side of the neck with 0.2 ml of SalN tumor cells (1x10⁶) (Baskar 1995).

Anti-murine CTLA-4 or an isotype matched control antibody (PharMingen, San Diego, CA, 200 ug/animal) were injected intraperitoneally on days 0, 4, 7 and 14 following the injection of tumor cells. Tumor measurements were taken during the course of the 3-4 week experiments using a Starrett SPC Plus
5 electronic caliper (Athol, MA) and tumor size was expressed as the surface area covered by tumor growth (mm²).

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model. As shown in Figure 18, animals treated with anti-CTLA-4 had a reduction in tumor growth as
10 compared to animals treated with an isotype control antibody. Accordingly, anti-murine CTLA4 mAbs are capable of inhibiting growth of a fibrosarcoma in a mouse tumor model.

It is expected that antibodies that are cross-reactive with murine CTLA-4 would perform similarly in the model. However, of the antibodies of the
15 invention that have been checked for cross-reactivity, none are cross-reactive with murine CTLA-4.

EXAMPLE 12

Tumor Animal Model

20

In order to further investigate the activity of antibodies in accordance with the invention, a xenograft SCID mouse model was designed to test the eradication of established tumors and their derived metastases. In the model, SCID mice are provided with grafted human T cells and are implanted with
25 patient-derived non-small cell lung cell (NSCL) or colorectal carcinoma (CC) cells. Implantation is made into the gonadal fat pads of SCID mice. The tumors are allowed to grow, and thereafter removed. The mice develop human-like tumor and liver metastases. Such a model is described in Bumpers et al *J. Surgical Res.* 61:282-288 (1996).

30

It is expected that antibodies of the invention will inhibit growth of tumors formed in such mice.

INCORPORATION BY REFERENCE

- All references cited herein, including patents, patent applications,
5 papers, text books, and the like, and the references cited therein, to the extent
that they are not already, are hereby incorporated herein by reference in their
entirety. In addition, the following references are also incorporated by
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EQUIVALENTS

5 The foregoing description and Examples detail certain preferred
embodiments of the invention and describes the best mode contemplated by the
inventors. It will be appreciated, however, that no matter how detailed the
foregoing may appear in text, the invention may be practiced in many ways and
the invention should be construed in accordance with the appended claims and
10 any equivalents thereof.

CLAIMSWHAT WE CLAIM IS:

- 5 1. An antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V_H3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or
10 framework sequences shown in Figure 2.
2. The antibody of Claim 1, wherein the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6,
15 SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70.
3. The antibody of Claim 1, further comprising a light chain
20 variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,
25 and SEQ ID NO:71.
4. The antibody of Claim 2, further comprising a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15,
30 SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ

ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,
and SEQ ID NO:71.

5 5. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:1 and a light chain variable amino acid sequence
comprising SEQ ID NO:14.

10 6. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:2 and a light chain variable amino acid sequence
comprising SEQ ID NO:15.

15 7. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:4 and a light chain variable amino acid sequence
comprising SEQ ID NO:17.

8. A isolated human monoclonal antibody that is capable of binding
to CTLA-4.

20 9. The antibody of Claim 8, wherein the antibody is capable of
competing for binding with CTLA-4 with an antibody selected from the group
consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,
11.7.1, 12.3.1.1, and 12.9.1.1.

25 10. The antibody of Claim 8, wherein the antibody possesses a
substantially similar binding specificity to CTLA-4 as an antibody selected from
the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

30 11. The antibody of Claim 8, wherein the antibody is selected from
the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

12. The antibody of Claim 8, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

13. The antibody of Claim 12, wherein the lower mammalian species
5 comprises mouse, rat, and rabbit.

14. The antibody of Claim 8, wherein the antibody is cross reactive with CTLA-4 from primates.

10 15. The antibody of Claim 14, wherein the primates comprise cynomolgous and rhesus monkeys.

16. The antibody of Claim 8, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than
15 about 100:1.

17. The antibody of Claim 16, wherein the selectivity is about 500:1 or greater.

20 18. The antibody of Claim 8, wherein a binding affinity of the antibody is about 10^{-9} M or greater.

19. The antibody of Claim 18, wherein a binding affinity of the antibody is about 10^{-10} M or greater.

25

20. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM.

21. The antibody of Claim 20, wherein the antibody inhibits binding
30 between CTLA-4 and B7-2 with an IC_{50} of lower than about 0.38 nM.

22. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater.

23. The antibody of Claim 22, wherein the antibody inhibits binding
5 between CTLA-4 and B7-1 with an IC_{50} of lower than about 0.50 nM.

24. The antibody of Claim 8, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

10 25. The antibody of Claim 24, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

26. The antibody of Claim 8, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater.

15

27. The antibody of Claim 26, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

28. The antibody of Claim 8, wherein the antibody induces IL-2
20 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater.

29. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml
25 or greater

30. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

30

31. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

5 32. A humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

10 33. The antibody of Claim 32, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

34. The antibody of Claim 33, wherein the lower mammalian species comprises mouse, rat, and rabbit.

15

35. The antibody of Claim 32, wherein the antibody is cross reactive with CTLA-4 from primates.

20 36. The antibody of Claim 35, wherein the primates comprise cynomolgous and rhesus monkeys.

37. The antibody of Claim 32, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1.

25

38. The antibody of Claim 37, wherein the selectivity is about 500:1 or greater.

39. The antibody of Claim 32, wherein a binding affinity of the
30 antibody is about 10^{-9} M or greater.

40. The antibody of Claim 39, wherein a binding affinity of the antibody is about 10^{-10} M or greater.

41. The antibody of Claim 32, wherein the antibody inhibits binding
5 between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM.

42. The antibody of Claim 41, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 0.38 nM.

10 43. The antibody of Claim 32, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater.

44. The antibody of Claim 43, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 0.50 nM.

15

45. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

46. The antibody of Claim 45, wherein the antibody enhances IL-2
20 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

47. The antibody of Claim 32, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater.

25 48. The antibody of Claim 47, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

49. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml
30 or greater.

50. The antibody of Claim 49, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml or greater

5 51. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

52. The antibody of Claim 50, wherein the antibody enhances IL-2
10 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

53. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded
15 by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2.

20 54. The antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

55. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded
25 by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties:

a binding affinity for CTLA-4 of about 10^{-9} or greater;

inhibits binding between CTLA-4 and B7-1 with an IC₅₀ of about
100 nM or lower;

30 inhibits binding between CTLA-4 and B7-2 with an IC₅₀ of about
100 nM or lower; and

enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

56. An antibody that binds to CTLA-4, comprising a heavy chain
5 amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked
in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected
from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3,
which antibody has the following properties:

a binding affinity for CTLA-4 of about 10^{-9} or greater;
10 inhibits binding between CTLA-4 and B7-1 with an IC_{50} of about
100 nM or lower;
inhibits binding between CTLA-4 and B7-2 with an IC_{50} of about
100 nM or lower; and
enhances cytokine production in an assay of human T cells by
15 500 pg/ml or greater.

57. A cell culture system for assaying T cell stimulation, comprising
a culture of human T cell blasts co-cultured with a Raji cell line.

20 58. The cell culture system of Claim 57, wherein the T cell blasts are
washed prior to culture with the Raji cell line.

59. An assay for measuring T cell stimulation, comprising:
providing a culture of human T cell blasts and a Raji cell line;
25 contacting the culture with an agent; and
measuring cytokine production by the culture.

60. The assay of Claim 59, wherein the T cell blasts are washed prior
to culture with the Raji cell line.

30

61. The assay of Claim 59, wherein the cytokine is IL-2.

62. The assay of Claim 59, wherein the cytokine is IFN- γ .

63. The assay of Claim 59, wherein cytokine production is measured in supernatant isolated from the culture.

5

64. The assay of Claim 59, wherein the agent is an antibody.

65. The assay of Claim 59, wherein the antibody binds to CTLA-4.

10 66. A functional assay for screening a moiety for T cell stimulatory function, comprising:

providing a culture of human T cell blasts and a Raji cell line;
contacting the culture with the moiety; and
assessing cytokine production by the culture.

15

67. The assay of Claim 66, wherein the T cell blasts are washed prior to culture with the Raji cell line.

68. The assay of Claim 66, wherein the cytokine is IL-2.

20

69. The assay of Claim 66, wherein the cytokine is IFN- γ .

70. The assay of Claim 66, wherein cytokine production is assessed in supernatant isolated from the culture.

25

71. The assay of Claim 66, wherein the moiety is an antibody.

72. The assay of Claim 66, wherein the antibody binds to CTLA-4.

30 73. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

74. The assay of Claim 73, wherein the T cell blasts are washed prior to culture with the Raji cell line.
- 5 75. The assay of Claim 73, wherein the cytokine is IL-2.
76. The assay of Claim 73, wherein the cytokine is IFN- γ .
77. The assay of Claim 73, wherein cytokine production is assessed
10 in supernatant isolated from the culture.
78. The assay of Claim 73, wherein the agent is an antibody.
79. The assay of Claim 73, wherein the antibody binds to CTLA-4.
15
80. A method for screening an agent for T cell stimulatory activity,
comprising:
contacting the agent with a cell culture comprising human T cell
blasts and a Raji cell line; and
20 assessing cytokine production by the culture.
81. The method of Claim 80, wherein the T cell blasts are washed
prior to culture with the Raji cell line.
- 25 82. The method of Claim 80, wherein the cytokine is IL-2.
83. The method of Claim 80, wherein the cytokine is IFN- γ .
84. The method of Claim 80, wherein cytokine production is
30 assessed in supernatant isolated from the culture.

85. The method of Claim 80, wherein the agent is an antibody that binds to CTLA-4.

86. An assay for measuring T cell stimulation, comprising:
5 providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;
contacting the culture with an agent; and
measuring cytokine production by the cell population.

10 87. The assay of Claim 86, wherein the cytokine is IL-2.

88. The assay of Claim 86, wherein cytokine production is measured in supernatant isolated from the cell population.

15 89. The assay of Claim 86, wherein the agent is an antibody.

90. The assay of Claim 86, wherein the antibody binds to CTLA-4.

20 91. A functional assay for screening a moiety for T cell stimulatory function, comprising:

providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;

25 contacting the culture with the moiety; and
assessing cytokine production by the cell population.

92. The assay of Claim 91, wherein the cytokine is IL-2.

30 93. The assay of Claim 91, wherein cytokine production is assessed in supernatant isolated from the cell population.

94. The assay of Claim 91, wherein the moiety is an antibody .
95. The assay of Claim 91, wherein the antibody binds to CTLA-4.
- 5 96. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.
- 10 97. The assay of Claim 96, wherein the cytokine is IL-2.
98. The assay of Claim 96, wherein cytokine production is assessed in supernatant isolated from the cell population.
- 15 99. The assay of Claim 96, wherein the agent is an antibody.
100. The assay of Claim 96, wherein the antibody binds to CTLA-4.
101. A method for screening an agent for T cell stimulatory activity,
20 comprising:
contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and
assessing cytokine production by the cell population.
- 25 102. The method of Claim 101, wherein the cytokine is IL-2.
103. The method of Claim 101, wherein cytokine production is assessed in supernatant isolated from the cell population.
- 30 104. The method of Claim 101, wherein the agent is an antibody that binds to CTLA-4.

Figure 1Figure 1A4.1.1 Heavy Chain DNA

ATGGAGTTTG	GGCTGAGCTG	GGTTTTCTC	GTTGCTCTT	TAAGAGGTGT	50
CCAGTGTCTAG	GTGCAGCTGG	TGGAGTCTGG	GGGAGGCGTG	GTCCAGCCTG	100
GGAGGTCCCT	GAGACTCTCC	TGTGTAGCGT	CTGGATTAC	CTTCAGTAGC	150
CATGGCATGC	ACTGGGTCCG	CCAGGCTCCA	GGCAAGGGGC	TGGAGTGGGT	200
GGCAGTTATA	TGGTATGATG	GAAGAAATAA	ATACTATGCA	GACTCCGTGA	250
AGGGCCGATT	CACCATCTCC	AGAGACAATT	CCAAGAACAC	GCTGTTTCTG	300
CAAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTGTATT	ACTGTGCGAG	350
AGGAGGTAC	TTCGTCTCTT	TTGACTACTG	GGGCCAGGGA	ACCCTGGTCA	400
CCGTCTCTC	AGCTCCACC	AAGGGCCCAT	CGGTCTTCCC	CCTGGCGCCC	450
TGCTCCAGGA	GCACCTCCGA	GAGCACAGCG	GCCCTGGGCT	GCCTGGTCAA	500
GGACTACTTC	CCCGAACC GG	TGACGGTGTC	GTGGAAC TCA	GGCGCTCTGA	550
CCAGCGGCGT	GCACACCTTC	CCAGCTGTCC	TACAGTCCTC	AGGACTCTAC	600
TCCCTCAGCA	GCGTGGTGAC	CGTGCCCTCC	AGCAACTTCG	GCACCCAGAC	650
CTACACCTGC	AACGTAGATC	ACAAGCCCAG	CAACACCAAG	GTGGACAAGA	700
CAGTTGAGCG	CAATGTTGT	GTGAGTGCC	CACCGTGCCC	AGCACCACCT	750
GTGGCAGGAC	CGTCAGTCTT	CCTCTTCCCC	CCAAAACCCA	AGGACACCCT	800
CATGATCTCC	CGGACCCCTG	AGGTCACGTG	CGTGGTGGTG	GACGTGAGCC	850
ACGAAGACCC	CGAGGTCCAG	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG	900
CATAATGCCA	AGACAAAGCC	ACGGGAGGAG	CAGTTCAACA	GCACGTTCCG	950
TGTGGTCAGC	GTCTCACC GG	TTGTGCACCA	GGACTGGCTG	AACGGCAAGG	1000
AGTACAAGTG	CAAGGTCTCC	AACAAAGGCC	TCCCAGCCCC	CATCGAGAAA	1050
ACCATCTCCA	AAACCAAAGG	GCAGCCCCGA	GAACCACAGG	TGTACACCCT	1100
CGCCCCATCC	CGGGAGGAGA	TGACCAAGAA	CCAGGTACAG	CTGACCTGCC	1150
TGGTCAAAGG	CTTCTACCCC	AGCGACATCG	CCGTGGAGTG	GGAGAGCAAT	1200
GGGCAGCCGG	AGAACAATA	CAAGACCACA	CCTCCCATGC	TGGACTCCGA	1250
CGGCTCCTTC	TTCTCTACA	GCAAGCTCAC	CGTGGACAAG	AGCAGGTGGC	1300
AGCAGGGGAA	CGTCTTCTCA	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC	1350
CACTACACGC	AGAAGAGCCT	CTCCCTGTCT	CCGGGTAAAT	GA	1392

(SEQ ID NO:27)

4.1.1 Heavy Chain Protein

MEFGLSWVFL	VALLRGVQCQ	VQLVESGGGV	VQGRSLRLS	CVASGFTFSS	50
HGMHWVRQAP	GKGLEWVAVI	WYDGRNKYYA	DSVKGRFTIS	RDNSKNTLFL	100
QMNSLRAEDT	AVYYCARGGH	FGPFDYWGQG	TLVTVSSAST	KGPSVFPLAP	150
CSRSTSESTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	PAVLQSSGLY	200
SLSSVTVPS	SNFGTQTYTC	NVDHKPSNTK	VDKTVERKCC	VECPPCPAPP	250
VAGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVQ	FNWYVDGVEV	300
HNAKTKPREE	QFNSTFRVVS	VLTVVHQDWL	NGKEYKCKVS	NKGLPAPIEK	350
TISKTKGQPR	EPQVYTLPPS	REEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	400
GQPENNYKTT	PPMLDSGGSF	FLYSKLTVDK	SRWQQGNVFS	CSVMHEALHN	450
HYTQKSLSL	PGK				463

(SEQ ID NO:1)

Figure 1Figure 1A (continued)4.1.1 Kappa Chain DNA

ATGGAAACCC	CAGCGCAGCT	TCTCTTCCTC	CTGCTACTCT	GGCTCCCAGA	50
TACCACCGGA	GAAATTGTGT	TGACGCAGTC	TCCAGGCACC	CTGTCTTTGT	100
CTCCAGGGGA	AAGAGCCACC	CTCTCCTGCA	GGGCCAGTCA	GAGTATTAGC	150
AGCAGCTTCT	TAGCCTGGTA	CCAGCAGAGA	CCTGGCCAGG	CTCCAGGCT	200
CCTCATCTAT	GGTGCATCCA	GCAGGGCCAC	TGGCATCCCA	GACAGGTTCA	250
GTGGCAGTGG	GTCTGGGACA	GACTTCACTC	TCACCATCAG	CAGACTGGAG	300
CCTGAAGATT	TTGCAGTGTA	TTACTGTCAG	CAGTATGGTA	CCTCACCCTG	350
GACGTTCCGC	CAAGGGACCA	AGGTGGAAAT	CAAACGAACT	GTGGCTGCAC	400
CATCTGTCTT	CATCTTCCCG	CCATCTGATG	AGCAGTTGAA	ATCTGGAAT	450
GCCTCTGTTG	TGTGCCTGCT	GAATAACTTC	TATCCCAGAG	AGGCCAAAGT	500
ACAGTGGAAG	GTGGATAACG	CCCTCCAATC	GGGTAAGTCC	CAGGAGAGTG	550
TCACAGAGCA	GGACAGCAAG	GACAGCACCT	ACAGCCTCAG	CAGCACCTG	600
ACGCTGAGCA	AAGCAGACTA	CGAGAAACAC	AAAGTCTACG	CCTGCGAAGT	650
CACCCATCAG	GGCCTGAGCT	CGCCCGTCAC	AAAGAGCTTC	AACAGGGGAG	700
AGTGTTAG					708

(SEQ ID NO:40)

4.1.1 Kappa Chain Protein

METPAQLLFL	LLLWLPDTTG	EIVLTQSPGT	LSLSPGERAT	LSCRASQSIG	50
SSFLAWYQQR	PGQAPRLLIY	GASSRATGIP	DRFSGSGSGT	DFTLTISRLE	100
PEDFAVYYCQ	QYGTSPWTFG	QGTKVEIKRT	VAAPSVFIFP	PSDEQLKSGT	150
ASVVCLLNNF	YPREAKVQWK	VDNALQSGNS	QESVTEQDSK	DSTYLSSTL	200
TLISKADYKX	KVYACEVTHQ	GLSSPVTKSF	NRGEC		235

(SEQ ID NO:14)

Figure 1Figure 1B4.8.1 Heavy Chain DNA

ATGGAGTTTG	GGCTGAGCTG	GGTTTTCTC	GTTGCTCTTT	TAAGAGGTGT	50
CCAGTGTTCAG	GTGCAGCTGG	TGGAGTCTGG	GGGAGGCGTG	GTCCAGCCTG	100
GGAGGTCCCT	GAGACTCTCC	TGTACAGCGT	CTGGATTAC	CTTCAGTAAC	150
TATGGCATGC	ACTGGGTCCG	CCAGGCTCCA	GGCAAGGGGC	TGGAGTGGGT	200
GGCAGTTATA	TGGTATGATG	GAAGTAATAA	AACTATGGA	GACTCCGTGA	250
AGGGCCGATT	CACCATCTCC	AGTGACAATT	CCAAGAACAC	GCTGTATCTG	300
CAAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTGTATT	ACTGTGCGAG	350
AGGAGAGAGA	CTGGGGTCCT	ACTTTGACTA	CTGGGGCCAG	GGAACCCTGG	400
TCACCGTCTC	CTCAGCCTCC	ACCAAGGGCC	CATCGGTCTT	CCCCCTGGCG	450
CCCTGCTCCA	GGAGCACCTC	CGAGAGCACA	GCGGCCCTGG	GCTGCCTGGT	500
CAAGGACTAC	TTCCCCGAAC	CGGTGACGGT	GTCGTGGAAC	TCAGGCGCTC	550
TGACCAGCGG	CGTGACACCC	TTCCCAGCTG	TCCTACAGTC	CTCAGGACTC	600
TACTCCCTCA	GCAGCGTGGT	GACCGTGCCC	TCCAGCAACT	TCGGCACCCA	650
GACCTACACC	TGCAACGTAG	ATCACAAGCC	CAGCAACACC	AAGGTGGACA	700
AGACAGTTGA	GCGCAAATGT	TGTGTCGAGT	GCCCACCGTG	CCCAGCACCA	750
CCTGTGGCAG	GACCGTCAGT	CTTCCTCTTC	CCCCAAAAC	CCAAGGACAC	800
CCTCATGATC	TCCCGGACCC	CTGAGGTCAC	GTGCGTGGTG	GTGGACGTGA	850
GCCACGAAGA	CCCCGAGGTC	CAGTTCAACT	GGTACGTGGA	CGGCGTGGAG	900
GTGCATAATG	CCAAGACAAA	GCCACGGGAG	GAGCAGTTCA	ACAGCACGTT	950
CCGTGTGGTC	AGCGTCCTCA	CCGTTGTGCA	CCAGGACTGG	CTGAACGGCA	1000
AGGAGTACAA	GTGCAAGGTC	TCCAACAAAG	GCCTCCACG	CCCCATCGAG	1050
AAAACCATCT	CCAAAACCAA	AGGGCAGCCC	CGAGAACCAC	AGGTGTACAC	1100
CCTGCCCCCA	TCCCGGAGG	AGATGACCAA	GAACCAGGTC	AGCCTGACCT	1150
GCCTGGTCAA	AGGCTTCTAC	CCCAGCGACA	TCGCCGTGGA	GTGGGAGAGC	1200
AATGGGAGC	CGGAGAACAA	CTACAAGACC	ACACCTCCCA	TGCTGGACTC	1250
CGACGGCTCC	TTCTTCCTCT	ACAGCAAGCT	CACCGTGGAC	AAGAGCAGGT	1300
GGCAGCAGGG	GAACGTCTTC	TCATGCTCCG	TGATGCATGA	GGCTCTGCAC	1350
AACCACTACA	CGCAGAAGAG	CCTCTCCCTG	TCTCCGGGTA	AATGA	1395

(SEQ ID NO:28)

4.8.1 Heavy Chain Protein

MEFGLSWVFL	VALLRGVQCQ	VQLVESGGGV	VQPGSLRLS	CTASGFTFSN	50
YGMHWVRQAP	GKGLEWVAVI	WYDGSNKHYG	DSVKGRFTIS	SDNSKNTLYL	100
QMNSLRAEDT	AVYYCARGER	LGSYFDYWQ	GTLTVSSAS	TKGPSVFPLA	150
PCSRSTSEST	AALGCLVKDY	FPEPVTVSWN	SGALTSGVHT	FPAVLQSSGL	200
YSLSSVVTVP	SSNFGTQTYT	CNVDHKPSNT	KVDKTVKRC	CVECPPCPAP	250
PVAGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSHEDPEV	QFNWYVDGVE	300
VHNAKTKPRE	EQFNSTFRV	SVLTVVHQDW	LNGKEYKCKV	SNKGLPAPIE	350
KTISKTKGQP	REPQVYTLPP	SREEMTKNQV	SLTCLVKGFY	PSDIAVEWES	400
NGQPENNYKT	TPPMLDSGGS	FFLYSKLTVD	KSRWQQGNVF	SCSVMHEALH	500
NHYTQKSLSL	SPGK				514

(SEQ ID NO:2)

Figure 1Figure 1B (continued)4.8.1 Kappa Chain DNA

ATGGAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA	50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT	100
CTCCAGGGGA AAGAGCCACC CTCTCCTGCA GGACCAGTGT TAGCAGCAGT	150
TACTTAGCCT GGTACCAGCA GAAACCTGGC CAGGCTCCCA GGCTCCTCAT	200
CTATGGTGCA TCCAGCAGGG CCACTGGCAT CCCAGACAGG TTCAGTGGCA	250
GTGGGTCTGG GACAGACTTC ACTCTACCA TCAGCAGACT GGAGCCTGAA	300
GATTTTGCAG TCTATTACTG TCAGCAGTAT GGCATCTCAC CCTTCACTTT	350
CGGCGGAGGG ACCAAGGTGG AGATCAAGCG AACTGTGGCT GCACCATCTG	400
TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAATCTGG AACTGCCTCT	450
GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG	500
GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTACAG	550
AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG	600
AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA	650
TCAGGGCCTG AGCTCGCCCC TCACAAAGAG CTTCAACAGG GGAGAGTGTT	700
AG	702

(SEQ ID NO:41)

4.8.1 Kappa Chain Protein

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRTSVSSS	50
YLAWYQKPG QAPRLLIYGA SSRATGIPDR FSGSGSGTDF TLTISRLEPE	100
DFAVYYCQY GISPFTFGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS	150
VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL	200
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC	233

(SEQ ID NO:15)

Figure 1Figure 1C4.14.3 Heavy Chain DNA

CCTGGGAGGT	CCCTGAGACT	CTCCTGTGCA	GCGTCTGGAT	TCACCTTCAG	50
TAGTCATGGC	ATCCACTGGG	TCCGCCAGGC	TCCAGGCAAG	GGGCTGGAGT	100
GGGTGGCAGT	TATATGGTAT	GATGGAAGAA	ATAAAGACTA	TGCAGACTCC	150
GTGAAGGGCC	GATTCACCAT	CTCCAGAGAC	AATTCCAAGA	AGACGCTGTA	200
TTTGCAAATG	AACAGCCTGA	GAGCCGAGGA	CACGGCTGTG	TATTACTGTG	250
CGAGAGTGGC	CCCACTGGGG	CCACTTGACT	ACTGGGGCCA	GGGAACCCTG	300
GTCACCGTCT	CCTCAGCCTC	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	350
GCCCTGCTCC	AGGAGCACCT	CCGAGAGCAC	AGCGGCCCTG	GGCTGCCTGG	400
TCAAGGACTA	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCT	450
CTGACCAGCG	GCGTGCACAC	CTTCCCAGCT	GTCTACAG		489

(SEQ ID NO:29)

4.14.3 Heavy Chain Protein

PGRSLRLSCA	ASGFTFSSHG	IHWVRQAPGK	GLEWVAVIWY	DGRNKDYADS	50
VKGRFTISR	NSKKTLYLQM	NSLRAEDTAV	YYCARVAPLG	PLDYWGQGT	100
VTVSSASTKG	PSVFPPLAPCS	RSTSESTAAL	GCLVKDYFPE	PVTVSWNSGA	150
LTSGVHTFPA	VLQ				163

(SEQ ID NO:3)

4.14.3 Kappa Chain DNA

GGCACCTGT	CTTTGTCTCC	AGGGGAAAGA	GCCACCCTCT	CCTGCAGGGC	50
CAGTCAGAGT	GTCAGCAGCT	ACTTAGCCTG	GTACCAGCAG	AAACCTGGCC	100
AGGCTCCCAG	ACTCCTCATC	TATGGTGCAT	CCAGCAGGGC	CACTGGCATC	150
CCAGACAGGT	TCAGTGGCAG	TGGGTCTGGG	ACAGACTTCA	CTCTCACCAT	200
CAGCAGACTG	GAGCCTGAGG	ATTTTGCAGT	GTATTACTGT	CAGCAGTATG	250
GTAGGTCACC	ATTCACCTTC	GGCCCTGGGA	CCAAAGTGGG	TATCAAGCGA	300
ACTGTGGCTG	CACCATCTGT	CTTCATCTTC	CCGCCATCTG	ATGAGCAGTT	350
GAAATCTGGA	ACTGCCTCTG	TTGTGTGCCT	GCTGAATAAC	TTCTATCCCA	400
GAGAGGCCAA	AGTACAG				417

(SEQ ID NO:42)

4.14.3 Kappa Chain Protein

GTLTSLSPGER	ATLSCRASQS	VSSYLAWYQQ	KPGQAPRLLI	YGASSRATGI	50
PDRFSGSGSG	TDFTLTISRL	EPEDFAVYYC	QQYGRSPFTF	GPGTKVDIKR	100
TVAAPSVFIF	PPSDEQLKSG	TASVVCLLNN	FYPREARKVQ		139

(SEQ ID NO:16)

Figure 1Figure 1D6.1.1 Heavy Chain DNA

ATGGAGTTTG	GGCTGAGCTG	GGTTTTCTC	GTTGCTCTTT	TAAGAGGTGT	50
CCAGTGTCTAG	GTGCAGCTGG	TGGAGTCTGG	GGGAGGCGTG	GTCGAGCCTG	100
GGAGGTCCCT	GAGACTCTCC	TGTACAGCGT	CTGGATTAC	CTTCAGTAGT	150
TATGGCATGC	ACTGGGTCCG	CCAGGCTCCA	GGCAAGGGGC	TGGAGTGGGT	200
GGCAGTTATA	TGGTATGATG	GAAGCAATAA	ACACTATGCA	GACTCCGCGA	250
AGGGCCGATT	CACCATCTCC	AGAGACAATT	CCAAGAACAC	GCTGTATCTG	300
CAAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTGTATT	ACTGTGCGAG	350
AGCCGGACTG	CTGGGTACT	TTGACTACTG	GGGCCAGGGA	ACCTTGGTCA	400
CCGTCTCTC	AGCCTCCACC	AAGGGCCCAT	CGGTCTTCCC	CCTGGCGCCC	450
TGCTCCAGGA	GCACCTCCGA	GAGCACAGCG	GCCCTGGGCT	GCCTGGTCAA	500
GGACTACTTC	CCCCAACCAG	TGACGGTGTC	GTGGAATCA	GGCGCTCTGA	550
CCAGCGGCGT	GCACACCTTC	CCAGCTGTCC	TACAGTCTTC	AGGACTCTAC	600
TCCCTCAGCA	AGCGTGGTGAC	CGTGCCCTCC	AGCAACTTCG	GCACCCAGAC	650
CTACACCTGC	AACGTAGATC	ACAAGCCCAG	CAACACCAAG	GTGGACAAGA	700
CAGTTGAGCG	CAAATGTTGT	GTCGAGTGCC	CACCGTGCCC	AGCACCACCT	750
GTGGCAGGAC	CGTCAGTCTT	CCTCTTCCCC	CCAAAACCCA	AGGACACCCT	800
CATGATCTCC	CGGACCCCTG	AGGTACCGTG	CGTGGTGGTG	GACGTGAGCC	850
ACGAAGACCC	CGAGGTCCAG	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG	900
CATAATGCCA	AGACAAAGCC	ACGGGAGGAG	CAGTCAACA	GCACGTTCCG	950
TGTGGTCAGC	GTCCTCACCG	TTGTGCACCA	GGACTGGCTG	AACGGCAAGG	1000
AGTACAAGTG	CAAGGTCTCC	AACAAAGGCC	TCCCAGCCCC	CATCGAGAAA	1050
ACCATCTCCA	AAACCAAAGG	GCAGCCCCGA	GAACCACAGG	TGTACACCCT	1100
GCCCCCATCC	CGGGAGGAGA	TGACCAAGAA	CCAGGTCAGC	CTGACCTGCC	1150
TGGTCAAAGG	CTTCTACCCC	AGCGACATCG	CCGTGGAGTG	GGAGAGCAAT	1200
GGGCAGCCGG	AGAACAACTA	CAAGACCACA	CCTCCCATGC	TGGACTCCGA	1250
CGGCTCCTTC	TTCTCTTACA	GCAAGCTCAC	CGTGACAAG	AGCAGGTGGC	1300
AGCAGGGGAA	CGTCTTCTCA	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC	1350
CACTACACGC	AGAAGAGCCT	CTCCCTGTCT	CCGGGTAAAT	GA	1392

(SEQ ID NO:30)

6.1.1 Heavy Chain Protein

MEFGLSWVFL	VALLRGVQCQ	VQLVESGGGV	VEPGRSLRLS	CTASGFTFSS	50
YGMHWVRQAP	GKGLEWVAVI	WYDGSNKHYA	DSAKGRFTIS	RDNSKNTLYL	100
QMNSLRAEDT	AVYYCARAGL	LGYFDYWGQG	TLVTVSSAST	KGPSVFPFLAP	150
CSRSTSESTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	PAVLQSSGLY	200
SLSSVVTVP	SNFGTQTYTC	NVDHKPSNTK	VDKTVERKCC	VECPPCPAPP	250
VAGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVQ	FNWYVDGVEV	300
HNAKTKPREE	QFNSTFRVVS	VLTVVHQDWL	NGKEYKCKVS	NKGLPAPIEK	350
TISKTKGQPR	EPQVYTLPPS	REEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	400
GQPENNYKTT	PPMLSDGSF	FLYSKLTVDK	SRWQQGNVFS	CSVMHEALHN	450
HYTQKSLSL	PGK				463

(SEQ ID NO:4)

Figure 1Figure 1D (continued)6.1.1 Kappa Chain DNA

<u>ATGGAAACCC CAGCGCAGCT</u>	TCTCTTCCTC	CTGCTACTCT	GGCTCCCAGA	50
TACCACCGGA	GAAATTGTGT	TGACGCAGTC	TCCAGGCACC	100
CTCCAGGGGA	AAGAGCCACC	CTCTCCTGTA	GGGCCAGTCA	150
AGCTACTTAG	CCTGGTACCA	ACAGAAACCT	GGCCAGGCTC	200
CATCTATGGT	GTATCCAGCA	GGGCCACTGG	CATCCCAGAC	250
GCAGTGGGTC	TGGGACAGAC	TTCACTCTCA	CCATCAGCAG	300
GAAGATTTTG	CAGTGATTA	CTGTCAGCAG	TATGGTATCT	350
TTTCGGCCCT	GGGACCAAAG	TGGATATCAA	ACGAACTGTG	400
CTGTCTTCAT	CTTCCCGCCA	TCTGATGAGC	AGTTGAAATC	450
TCTGTTGTGT	GCCTGCTGAA	TAACTTCTAT	CCCAGAGAGG	500
GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACTCCCAG	550
CAGAGCAGGA	CAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	600
CTGAGCAAAG	CAGACTACGA	GAAACACAAA	GTCTACGCCT	650
CCATCAGGGC	CTGAGCTCGC	CCGTCACAAA	GAGCTTCAAC	700
GTTAG				705

(SEQ ID NO:43)

6.1.1 Kappa Chain Protein

METPAQLLFL	LLLWL PDTTG	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	50
SYLAWYQQKP	GQAPRPLIYG	VSSRATGIPD	RFSGSGSGTD	FTLTISRLEP	100
EDFAVYYCQQ	YGISPFTFGP	GTKVDIKRTV	AAPSVFIFPP	SDEQLKSGTA	150
SVVCLLNIFY	PREAKVQWKV	DNALQSGNSQ	ESVTEQDSKD	STYSLSSTLT	200
LSKADYEKHK	VYACEVTHQG	LSSPVTKSFN	RGEC		234

(SEQ ID NO:17)

Figure 1Figure 1E3.1.1 Heavy Chain DNA

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG	50
ATTCACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA	100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC	150
TATGCAGACT CCGTGAAGGG CCGATTCACT ATCTCCAGAG ACAATTCCAA	200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG	250
TGTATTACTG TGCAGAGAGG GCCCGTATAA TAACCCCTTG TATGGACGTC	300
TGGGGCCAAG GGACCACGGT CACCGTCTCC TCAGCCTCCA CCAAGGGCCC	350
ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG	400
CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG	450
TCGTGGAACCT CAGGCGCTCT GACCAGCGGC GTGCACACCT TCCCAGCTGT	500
CCTACAG	507

(SEQ ID NO:31)

3.1.1 Heavy Chain Protein

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY	50
YADSVKGRFT ISRDNKNTL YLQMNSLRAE DTAVYYCARG ARIITPCMDV	100
WGQGTITVTS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV	150
SWNSGALTSG VHTFPAVLQ	169

(SEQ ID NO:5)

3.1.1 Kappa Chain DNA

CAGTCTCCAT CCTCCCTGTC TGCATCTGTA GGAGACAGAG TCACCATCAC	50
TTGCCGGGCA AGTCAGAGCA TTAACACCTA TTTAATTGG TATCAGCAGA	100
AACCAGGGAA AGCCCCTAAC TTCCTGATCT CTGCTACATC CATTTTGCAA	150
AGTGGGGTCC CATCAAGGTT CCGTGCCAGT GGCTCTGGGA CAAATTTTAC	200
TCTCACCATC AACAGTCTTC ATCCTGAAGA TTTTGCAACT TACTACTGTC	250
AACAGAGTTA CAGTACCCCA TTCACTTTTCG GCCCTGGGAC CAAAGTGGAT	300
ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA	350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT	400
TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA	450
TCGGGTAA	458

(SEQ ID NO:44)

3.1.1 Kappa Chain Protein

QSPSSLSASV GDRVITICRA SQSINTYLIW YQKPKGKAPN FLISATSILO	50
SGVPSRFRGS GSGTNFTLTI NSLHPEDFAT YYCQSYSTP FTFPGTKVD	100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ	150
SG	152

(SEQ ID NO:18)

Figure 1Figure 1F4.10.2 Heavy Chain DNA

GGCGTGGTCC	AGCCTGGGAG	GTCCCTGAGA	CTCTCCTGTG	TAGCGTCTGG	50
ATTTCATCTTC	AGTAGTCATG	GCATCCACTG	GGTCCGCCAG	GCTCCAGGCA	100
AGGGGCTGGA	GTGGGTGGCA	GTTATATGGT	ATGATGGAAG	AAATAAAGAC	150
TATGCAGACT	CCGTGAAGGG	CCGATTCACC	ATCTCCAGAG	ACAATTCCAA	200
GAACACGCTG	TATTTGCAAA	TGAACAGCCT	GAGAGCCGAG	GACACGGCTG	250
TGTATTACTG	TGCGAGAGTG	GCCCCACTGG	GGCCACTTGA	CTACTGGGGC	300
CAGGGAACCC	TGGTCACCGT	CTCCTCAGCC	TCCACCAAGG	GCCCATCGGT	350
CTTCCCCCTG	GCGCCCTGCT	CCAGGAGCAC	CTCCGAGAGC	ACAGCGGCCC	400
TGGGCTGCCT	GGTCAAGGAC	TACTTCCCCG	AACCGGTGAC	GGTGTCTGTG	450
AACTCAGGCG	CTCTGACCAG	CGGCGTGCAC	ACCTTCCCAG	CTGTCTTACA	500
G					501

(SEQ ID NO:32)

4.10.2 Heavy Chain Protein

GVVQPGRSRLR	LSCVASGFIF	SSHGIHWVRQ	APGKGLEWVA	VIWYDGRNKD	50
YADSVKGRFT	ISRDNKXNTL	YLOMNSLRAE	DTAVYYCARV	APLGPLDYWG	100
QGTTLVTVSSA	STKGPSVFPL	APCSRSTSES	TAALGCLVKD	YFPEPVTVSW	150
NSGALTSGVH	TFPAVLQ				167

(SEQ ID NO:6)

4.10.2 Kappa Chain DNA

TCTCCAGGCA	CCCTGTCTTT	GTCTCCAGGG	GAAAGAGCCA	CCCTCTCCTG	50
CAGGGCCAGT	CAGAGTATTA	GCAGCAATTT	CTTAGCCTGG	TACCAGCAGA	100
AACCTGGCCA	GGCTCCCAGG	CTCCTCATCT	ATCGTCCATC	CAGCAGGGCC	150
ACTGGCATCC	CAGACAGTTT	CAGTGGCAGT	GGGTCTGGGA	CAGACTTCAC	200
TCTCACCATC	AGCAGACTGG	AGCCTGAGGA	TTTTGCATTA	TATTACTGTC	250
AGCAGTATGG	TACGTACCA	TTCACTTTCG	GCCCTGGGAC	CAAAGTGGAT	300
ATCAAGCGAA	CTGTGGCTGC	ACCATCTGTC	TTCATCTTCC	CGCCATCTGA	350
TGAGCAGTTG	AAATCTGGAA	CTGCCTCTGT	TGTGTGCCTG	CTGAATAACT	400
TCTATCCCAG	AGAGGCCAAA	GTACAG			426

(SEQ ID NO:45)

4.10.2 Kappa Chain Protein

SPGTLSSLSPG	ERATLSCRAS	QSISSNFLAW	YQKPGQAPR	LLIYRPSSRA	50
TGIPDSFSGS	GSGETDFTLI	SRLEPEDFAL	YYCQYGTSP	FTFGPGTKVD	100
IKRTVAAPSV	FIFPPSDEQL	KSGTASVVCL	LNNFYPREAK	VQ	142

(SEQ ID NO:19)

Figure 1Figure 1G2.1.3 Heavy Chain DNA

TCGGGCCCAG	GACTGGTGAA	GCCTTCACAG	ATCCTGTCCC	TCACCTGCAC	50
TGCTCTGGT	GGCTCCATCA	GCAGTGGTGG	TCCTACTGG	AGCTGGATCC	100
GCCAGCACCC	AGGGAAGGGC	CTGGAGTGA	TTGGGTACAT	CTATTACATT	150
GGGAACACCT	ACTACAACCC	GTCCCTCAAG	AGTCGAGTTA	CCATATCAGT	200
AGACACGTCT	AAGAACCAGT	TCTCCCTGAA	GCTGAGCTCT	GTGACTGCCG	250
CGGACACGGC	CGTGATTAT	TGTGCGAGAG	ATAGTGGGGA	CTACTACGGT	300
ATAGACGTCT	GGGGCCAAGG	GACCACGGTC	ACCGTCTCCT	CAGCTTCCAC	350
CAAGGGCCCA	TCCGTCTTCC	CCCTGGCGCC	CTGCTCCAGG	AGCACCTCCG	400
AGAGCACAGC	CGCCCTGGGC	TGCCTGGTCA	AGGACTACTT	CCCCGAACCG	450
GTGACGGTGT	CGTGAACTC	AGGCGCCCTG	ACCAGCGGCG	TGCACACCTT	500
CCCGGCTGTC	CTACAA				516

(SEQ ID NO:33)

2.1.3 Heavy Chain Protein

SGPGLVKPSQ	ILSLTCTVSG	GSISSGGHYW	SWIRQHPGKG	LEWIGYIYYI	50
GNTYYNPSLK	SRVTISVDTS	KNQFSLKLSS	VTAADTAVYY	CARDSGDYYG	100
IDVWGQGTTV	TVSSASTKGP	SVFPLAPCSR	STSESTAALG	CLVKDYFPEP	150
VTVSWNSGAL	TSGVHTFPAV	LQ			172

(SEQ ID NO:7)

2.1.3 Kappa Chain DNA

TCTCCAGACT	TTCAGTCTGT	GACTCCAAAG	GAGAAAGTCA	CCATCACCTG	50
CCGGGCCAGT	CAGAGCATTG	GTAGTAGCTT	ACATTGGTAT	CAGCAGAAAC	100
CAGATCAGTC	TCCAAAGCTC	CTCATCAAGT	ATGCTTCCCA	GTCCCTTCTCT	150
GGGGTCCCT	CGAGGTTTCA	TGGCAGTGA	TCTGGGACAG	ATTTCAACCCT	200
CACCATCAAT	AGCCTGGAAG	CTGAAGATGC	TGCAACGTAT	TACTGTCATC	250
AGAGTAGTAG	TTTACCGCTC	ACTTTCGGCG	GAGGGACCAA	GGTGGAGATC	300
AAACGAACTG	TGCTGCACC	ATCTGTCTTC	ATCTTCCCGC	CATCTGATGA	350
GCAGTTGAAA	TCTGGAAGT	CCTCTGTGT	GTGCCTGCTG	AATAACTTCT	400
ATCCAGAGA	GGCCAAAGTA	CAGTGGAAGG	TGGATAACGC	CCTCCAATCG	450
GGTAACTCCC	AGGAG				465

(SEQ ID NO:46)

2.1.3 Kappa Chain Protein

SPDFQSVTPK	EKVTITCRAS	QSIGSSLHWY	QOKPDQSPKL	LIKYASQSFS	50
GVPSRFGSG	SGTDFTLTIN	SLEAEDAATY	YCHQSSSLPL	TFGGGKVEI	100
KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL	NNFYPREAKV	QWKVDNALQS	150
GNSQE					155

(SEQ ID NO:20)

Figure 1Figure 1H4.13.1 Heavy Chain DNA

CCTGGGAGGT CCCTGAGACT CTCCTGTGCA GCGTCTGGAT TCACCTTCAG	50
TAGTCATGGC ATCCACTGGG TCCGCCAGGC TCCAGGCAAG GGGCTGGAGT	100
GGGTGGCAGT TATATGGTAT GATGGAAGAA ATAAAGACTA TGCAGACTCC	150
GTGAAGGGCC GATTCAACCAT CTCCAGAGAC AATTCCAAGA ACACGCTGTA	200
TTTGCAAATG AACAGCCTGA GAGCCGAGGA CACGGCTGTG TATTACTGTG	250
CGAGAGTGGC CCCACTGGGG CCACCTGACT ACTGGGGCCA GGAACCCCTG	300
GTCACCGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC	350
GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCGGCCCTG GGCTGCCTGG	400
TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCT	450
CTGACCAGC	459

(SEQ ID NO:34)

4.13.1 Heavy Chain Protein

PGRSLRLSCA ASGFTFSSHG IHVVRQAPGK GLEWVAVIY DGRNKDYADS	50
VKGRFTISRDKSKNTLYLQM NSLRSEDYAV YYCARVAPLG PLDYWGQGL	100
VTSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA	150
LTS	153

(SEQ ID NO:8)

4.13.1 Kappa Chain DNA

CAGTCTCCAG GCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC	50
CTGCAGGGCC AGTCAGAGTG TCAGCAGCTA CTTAGCCTGG TACCAGCAGA	100
AACCTGGCCA GGCTCCCAGG CTCCTCATCT ATGGTGCATC CAGCAGGGCC	150
ACTGGCATCC CAGACAGGTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC	200
TCTCACCATC AGCAGACTGG AGCCTGAGGA TTTTGCAGTG TATTACTGTC	250
AACAGTATGG TAGGTCACCA TTCACCTTCG GCCCTGGGAC CAAAGTAGAT	300
ATCAAGCGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA	350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT	400
TCTATCCCAG AGAGGCCAAA GTACAGTGGA AAGGTGGATA	440

(SEQ ID NO:47)

4.13.1 Kappa Chain Protein

QSPGTLSP GERATLSCRA SOSVSSYLAW YQKPGQAPR LLIYGASSRA	50
TGIPDRFSGS GSGTDFTLTI SRLEPEDFAV YYCQYGRSP FTFGPSTKVD	100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKGG	146

(SEQ ID NO:21)

Figure 1Figure 1I11.2.1 Heavy Chain DNA

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG	50
ATTCACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA	100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC	150
TATGCAGACT CCGTGAAGGG CCGATTCACC ATCTCCAGAG ACAATTCCAA	200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG	250
TGTATTACTG TCGAGAGAT CCGAGGGGAG CTACCCTTTA CTACTACTAC	300
TACCGGTKGG ACGTCTGGGG CCAAGGGACC ACGGTCACCG TCTCCTCAGC	350
CTCCACCAAG GGCCCATCGG TCTTCCCCCT GCGGCCCTGC TCCAGGAGCA	400
CCTCCGAGAG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC	450
GAACCGGTGA CGGTGTCTGT GAACTCAGGC GCTCTGACCA GCGGCGTGCA	500
CAC	503

(SEQ ID NO:35)

11.2.1 Heavy Chain Protein

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY	50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARD PRGATLYYYY	100
YRXDVWGQGT TTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP	150
EPVTVSWNSG ALTSGVH	

(SEQ ID NO:9)

11.2.1 Kappa Chain DNA

CCATCCTCCC TGTCTGCATC TGTAGGAGAC AGAGTCACCA TCACTTGCCG	50
GGCAAGTCAG AGCATTAAACA GCTATTAGA TTGGTATCAG CAGAAACCAG	100
GGAAAGCCCC TAAACTCCTG ATCTATGCTG CATCCAGTTT GCAAAGTGGG	150
GTCCCATCAA GGTTCAGTGG CAGTGGATCT GGGACAGATT TCACTCTCAC	200
CATCAGCAGT CTGCAACCTG AAGATTTTGC AACTTACTAC TGTCAACAGT	250
ATTACAGTAC TCCATTCACT TTCGGCCCTG GGACCAAAGT GGAAATCAAA	300
CGAACTGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA	350
GTGAAATCT GGAAGTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC	400
CCAGAGAGGC CAAAGTA	417

(SEQ ID NO:48)

11.2.1 Kappa Chain Protein

PSSLSASVGD RVTITCRASQ SINSYLDWYQ QKPGKAPKLL IYAASSLQSG	50
VPSRFGSGS GTDFTLTISS LQPEDFATYY CQYYSTPFT FGPGTKVEIK	100
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKV	139

(SEQ ID NO:22)

Figure 1Figure 1J11.6.1 Heavy Chain DNA

GGCGTGGTCC	AGCCTGGGAG	GTCCCTGAGA	CTCTCCTGTG	CAGCGTCTGG	50
ATTACCTTC	AGTAGCTATG	GCATGCACTG	GGTCCGCCAG	GCTCCAGGCA	100
AGGGGCTGGA	GTGGGTGGCA	GTTATATGGT	ATGATGGAAG	TCATAAATAC	150
TATGCAGACT	CCGTGAAGGG	CCGATTCACC	ATCTCCAGAG	ACAATTCCAA	200
GAACACGCTG	TATCTGCAAA	TGAACAGCCT	GAGAGCCGAG	GACACGGCTG	250
TGTATTACTG	TGCGAGAGGC	GCTGTAGTAG	TACCAGCTGC	TATGGACGTC	300
TGGGGCCAAG	GGACCACGGT	CACCGTCTCC	TCAGCCTCCA	CCAAGGGCCC	350
ATCGGTCTTC	CCCCTGGCGC	CCTGCTCCAG	GAGCACCTCC	GAGAGCACAG	400
CGGCCCTGGG	CTGCCTGGTC	AAGGACTACT	TCCCCGAACC	GGTGACGGTG	450
T					451

(SEQ ID NO:36)

11.6.1 Heavy Chain Protein

GVVQPGRS LR	LSCAASGFTF	SSYGMHWVRQ	APKGLEWVA	VIWYDGS HKY	50
YADSVKGRFT	ISRDN SKNTL	YLOMNSLRAE	DTAVYYCARG	AVVVPAAMDV	100
WGQGTITVTS	SASTKGPSVF	PLAPCSRSTS	ESTAALGCLV	KDYFPEPVTV	150
S					151

(SEQ ID NO:10)

11.6.1 Kappa Chain DNA

ACCCAGTCTC	CATCCTCCCT	GTCTGCATCT	G TAGGAGACA	GAGTCACCAT	50
CACTTGCCGG	GCAAGTCAGA	ACATTAGCAG	GTATTTAAAT	TGGTATCAAC	100
AGAAACCAGG	GAAAGCCCCT	AAGTTCCTGA	TCTATGTTGC	ATCTATTTTG	150
CAAAGTGGGG	TCCCATCAGG	GTTCAGTGCC	AGTGGATCTG	GGCCAGATTT	200
CACTCTNACC	ATCAGCAGTC	TGCAACCTGA	AGATTTTGCA	ACTTACTACT	250
GTCAACAGAG	TTACAGTACC	CCATTCACCT	TCGGCCCTGG	GACCAAAGTG	300
GATATCAAAC	GAAGTGTGGC	TGCACCATCT	GTCTTCATCT	TCCCGCCATC	350
TGATGAGCAG	TTGAAATCTG	GAAGTGCCTC	TGTTGTGTGC	CTGCTGAATA	400
AC					402

(SEQ ID NO:49)

11.6.1 Kappa Chain Protein

TQSPSSLSAS	VGDRVITICR	ASQNISRYLN	WYQQKPGKAP	KFLIYVASIL	50
QSGVPSGFS A	SGSGPDFTLT	ISSLPEDFA	TYQCQSYST	PFTFGPGTKV	100
DIKRTVAAPS	VFIFPPSDEQ	LKSGTASVVC	LLNN		134

(SEQ ID NO:23)

Figure 1Figure 1K11.7.1 Heavy Chain DNA

GTGGTCCAGC CTGGGAGGTC CCTGAGACTC TCCTGTGCAG CGTCTGGATT	50
CACCTTCAGT AGCNGTGGCA TGCCTGGGT CCGCCAGGCT CCAGGCAAGG	100
GGCTGGAGTG GGTGGCAGTT ATATGGTCTG ATGGAAGTCA TAAATACTAT	150
GCAGACTCCG TGAAGGGCCG ATTCACCATC TCCAGAGACA ATTCCAAGAA	200
CACGCTGTAT CTGCAAATGA ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT	250
ATTACTGTGC GAGAGGAACT ATGATAGTAG TGGGTACCCT TGACTIONTGG	300
GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC	350
GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG AGCACAGCGG	400
CCCTGGGCTG CCTGTCAAG GACTACTTCC CCGAACCG	438

(SEQ ID NO:37)

11.7.1 Heavy Chain Protein

VVQPGRSLRL SCAASGFTFS SXGMHWVROA PGKGLEWVAV IWSDGSHKYY	50
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGT MIVVGTLIDYV	100
QGTLVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEP	146

(SEQ ID NO:11)

11.7.1 Kappa Chain DNA

ACCCAGTCTC CATCCTCCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT	50
CACTTGCCGG GCAAGTCAGA GCATTGCAA CTATTTAAAT TGGTATCAGC	100
AGAAACCAGG AAAAGCCCCT AGGGTCCTGA TCTATGCTGC ATCCAGTTTG	150
CAAGGTGGGG TCCCGTCAAG GTTCAGTGGC AGTGGATCTG GGACAGATTG	200
CACTCTCACC ATCAGCAGTC TGCAACCTGA AGATTTTGCA ACTTACTACT	250
GTCAACAGAG TTAACTACC CCATTCACTT TCGGCCCTGG GACCAGAGTG	300
GATATCGAAC GAACTGTGGC TGCAACCATCT GTCTTCATCT TCCCGCCATC	350
TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA	400
ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCTAT	450
T	451

(SEQ ID NO:50)

11.7.1 Kappa Chain Protein

TQSPSSLSAS VGDRVTITCR ASQSICNYLN WYQQKPGKAP RVLIIYAASSL	50
QGGVPSRFSG SSGSIDCTLT ISSLOPEDFA TYYCQSYIT PFTFGPGTRV	100
DIERTVAAPS VFIFPPSDEQ LKSGTASVVC LLNNFYPREA KVQWKVDNAY	150

(SEQ ID NO:24)

Figure 1Figure 1L12.3.1 Heavy Chain DNA

TCCTGTGCAG CGTCTGGATT CACCTTCAGT TACTATGGCG

TCTGGGGGAG GCGTGGTCCA GCCTGGGAGG TCCCTGAGAC TCTCCTGTGC 50
 AGCGTCTGGA TTCACCTTCA GTAGCTATGG CGTGCACTGG GTCCGCCAGG 100
 CTCCAGGCAA GGGGCTGGAG TGGGTGGCAG TTATATGGTA TGATGGAAGT 150
 AATAAATACT ATGCAGACTC CGTGAAGGGC CGATTACCA TCTCCAGAGA 200
 CAATTCCAAG AGCAGCTGT ATCTGCAAAT GAACAGCCTG AGAGCCGAGG 250
 ACACGGCTGT GTATTATTGT GCGAGAGACT CGTATTACGA TTTTGGGAGT 300
 GGTGCGGGCG GTATGGACGT CTGGGGCCAA GGGACCACGG TCACCGTCTC 350
 CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCG CCCTGCTCCA 400
 GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT CAAGGACTAC 450
 TTCCCCGAAC CCGTGACGGT GTCGTGGAAC TCAGGCGCTC TGACCAGCGG 500
 CGTGCAACACC TTCCCAGCTG TC 522

(SEQ ID NO:38)

12.3.1.1 Heavy Chain Protein

SGGGVVQPGR SLRLSCAASG FTFSSYGVHW VRQAPGKGLE WVAVIWDGS 50
 NKYYADSVKG RFTISRDN SK STLYLQMN SL RAEDTAVYYC ARDSYYDFWS 100
 GRGGMDVWGQ GTTIVTSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY 150
 FPEPVTVSWN SGALTSGVHT FPAV 174

(SEQ ID NO:12)

12.3.1.1 Kappa Chain DNA

CCACTCTCCC TGCCCGTCAC CCTTGGACAG CCGGCCTCCA TCTCCTGCAG 50
 GTCTAGTCAA AGCCTCGTAT ACAGTGATGG AAACACCTAC TTGAATTGGT 100
 TTCAGCAGAG GCCAGGCCAA TCTCCAAGGC GCCTAATTTA TAAGGTTTCT 150
 AACTGGGACT CTGGGGTCCC AGACAGATTC AGCGGCAGTG GGTGAGGCAC 200
 TGATTTCACA CTGAAAATCA GCAGGGTGA GGTGAGGAT GTTGGGGTTT 250
 ATTACTGCAT GCAAGGTTCA CACTGGCCTC CGACGTTTCG CCAAGGGACC 300
 AAGGTGGA AA TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC 350
 GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCCTCTGTT GTGTGCCTGC 400
 TGAATAACTT CTATCCCAC 419

(SEQ ID NO:51)

12.3.1.1 Kappa Chain Protein

PLSLPVTLGQ PASISCRSSQ SLVYSDGNTY LNWFGQRPQ SPRLRIYKVS 50
 NWD SGVPDRF SGSGSGTDFT LKISRVEAD VGVYYCMQGS HWPPTFGQGT 100
 KVEIKRTVAA PSVFIFPPSD EQLKSGTASV VCLLNNFYP 139

(SEQ ID NO:25)

Figure 1Figure 1M12.9.1.1 Heavy Chain DNA

GTCCAGCCTG	GGAGGTCCCT	GAGACTCTCC	TGTGCAGCGT	CTGGATTCAC	50
CTTCAGTAAC	TATGCCATGC	ACTGGGTCCG	CCAGGCTCCA	GGCAAGGGGC	100
TGGAGTGGGT	GGTAGTTATT	TGGCATGATG	GAAATAATAA	ATACTATGCA	150
GAGTCCGTGA	AGGGCCGATT	CACCATCTCC	AGAGACAATT	CCAAGAACAC	200
GCTGTATCTG	CAAAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTATATT	250
ACTGTGCGAG	AGATCAGGGC	ACTGGCTGGT	ACGGAGGCTT	TGACTTCTGG	300
GGCCAGGGAA	CCCTGGTCA	CGTCTCCTCA	GCCTCCACCA	AGGGCCCATC	350
GGTCTTCCCC	CTGGCGCCCT	GCTCCAGGAG	CACCTCCGAG	AGCACAGCGG	400
CCCTGGGCTG	CCTGGTCAAG	GACTACTTCC	CCGAACCGGT	GACGGTGTCC	450
TGGAATCAG	GCGCTCTGAC	CAGCGGCGTG	CACACCTTCC		490

(SEQ ID NO:39)

12.9.1.1 Heavy Chain Protein

VQPGRSLRLS	CAASGFTFSN	YAMHWVRQAP	GKGLEWVVVI	WHDGNNKYIA	50
ESVKGRFTIS	RDNSKNTLYL	QMNSLRAEDT	AVYYCARDQG	TGWYGGFDWF	100
GQGTLLTVSS	ASTKGPSVFP	LAPCSRSTSE	STAALGCLVK	DYFPEPVTVS	150
WNSGALTSKV	HTF				163

(SEQ ID NO:13)

12.9.1.1 Kappa Chain DNA

CCTGGAGAGC	CGGCTTCCAT	CTCTTGCAGG	TCTAGTCAGA	GCCTCCTGCA	50
TAGTAATGGA	TACAACTATT	TGGATTGGTA	CCTGCAGAAG	CCAGGACAGT	100
CTCCACAGCT	CCTGATCTAT	TTGGGTTCTA	ATCGGGCCTC	CGGGGTCCCT	150
GACAGGTTC	GTGGCAGTGG	ATCAGGCACA	GATTTTACAC	TGAAACTCAG	200
CAGAGTGGAG	GCTGAGGATG	TTGGGGTTTA	TTACTGCATG	CAAGCTCTAC	250
AAACTCCTCT	CACCTTCGGC	GGAGGGACCA	AGGTGGAGAT	CAAACGAACT	300
GTGGCTGCAC	CATCTGTCTT	CATCTTCCCG	CCATCTGATG	AGCAGTTGAA	350
ATCTGGAAGT	GCCTCTGTTG	TGTGCCTGCT	GAATAACTTC	TATCCCAGAR	400
AGGCCAAAGT	ACATTCCAT				419

(SEQ ID NO:52)

12.9.1.1 Kappa Chain Protein

PGEPASISCR	SSQSLHSNG	YNYLDWYLQK	PGQSPQLLIY	LGSNRASGVP	50
DRFSGSGSGT	DFTLKLSRVE	AEDVGVYYCM	QALQTPLEFG	GGTKVEIKRT	100
VAAPSVFIFP	PSDEQLKSGT	ASVVCCLNNF	YPR		133

(SEQ ID NO:26)

Figure 2

CDR	DP50	3.1.1	4.1.1	4.8.1	4.10.2	4.13.1	4.14.3	6.1.1	11.2.1	11.6.1	11.7.1	12.3.1.1	12.9.1.1
	G	G	G	G	G			G	G	G		G	
	V	V	V	V	V			V	V	V	V	V	
	V	V	V	V	V			V	V	V	V	V	V
	Q	Q	Q	Q	Q			E	Q	Q	Q	Q	Q
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	L	L	L	L	L	L	L	L	L	L	L	L	L
	S	S	S	S	S	S	S	S	S	S	S	S	S
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	V	T	V	A	A	T	A	A	A	A	A
	A	A	A	A	A	A	A	A	A	A	A	A	A
	S	S	S	S	S	S	S	S	S	S	S	S	S
	G	G	G	G	G	G	G	G	G	G	G	G	G
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	I	T	T	T	T	T	T	T	T
CDR1	F	F	F	F	F	F	F	F	F	F	F	F	F
	S	S	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	N	S	S	S	S	S	S	S	S	N
	Y	Y	H	Y	H	H	H	Y	Y	Y	C	Y	Y
	G	G	G	G	G	G	G	G	G	G	G	G	A
	M	M	M	M	I	I	I	M	M	M	M	V	M
	H	H	H	H	H	H	H	H	H	H	H	H	H
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	R	R	R	R	R	R	R	R	R	R	R	R	R
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	A	A	A	A	A	A	A	A	A	A	A	A	A
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	L	L	L	L	L	L	L	L	L	L	L	L	L
	E	E	E	E	E	E	E	E	E	E	E	E	E
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	A	A	A	A	A	A	A	A	A	A	A	A	V
	V	V	V	V	V	V	V	V	V	V	V	V	V
	I	I	I	I	I	I	I	I	I	I	I	I	I
	W	W	W	W	W	W	W	W	W	W	W	W	W
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	S	Y	H
	D	D	D	D	D	D	D	D	D	D	D	D	D
CDR2	G	G	G	G	G	G	G	G	G	G	G	G	G
	S	S	R	S	R	R	R	S	S	S	S	S	N
	N	N	N	N	N	N	N	N	N	H	H	N	N
	K	K	K	K	K	K	K	K	K	K	K	K	K
	Y	Y	Y	H	D	D	D	H	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	A	A	A	G	A	A	A	A	A	A	A	A	A
	D	D	D	D	D	D	D	D	D	D	D	D	E
	S	S	S	S	S	S	S	S	S	S	S	S	S
	V	V	V	V	V	V	V	A	V	V	V	V	V
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	T	T	T	T	T	T	T	T	T
	I	I	I	I	I	I	I	I	I	I	I	I	I
	S	S	S	S	S	S	S	S	S	S	S	S	S
	R	R	R	S	R	R	R	R	R	R	R	R	R

Figure 2

CDR	DP50	3.1.1	4.1.1	4.8.1	4.10.2	4.13.1	4.14.3	6.1.1	11.2.1	11.6.1	11.7.1	12.3.1.1	12.9.1.1
	D	D	D	D	D	D	D	D	D	D	D	D	D
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	K	K	K	K	K	K	K	K	K	K	K	K	K
	N	N	N	N	N	N	K	N	N	N	N	S	N
	T	T	T	T	T	T	T	T	T	T	T	T	T
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Y	Y	F	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	M	M	M	M	M	M	M	M	M	M	M	M	M
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	A	A	A	A	A	A	A	A	A	A	A	A	A
	E	E	E	E	E	E	E	E	E	E	E	E	E
	D	D	D	D	D	D	D	D	D	D	D	D	D
	T	T	T	T	T	T	T	T	T	T	T	T	T
	A	A	A	A	A	A	A	A	A	A	A	A	A
	V	V	V	V	V	V	V	V	V	V	V	V	V
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	A	A	A	A	A	A	A	A	A	A	A
	R	R	R	R	R	R	R	R	R	R	R	R	R
		G	G	G	V	V	V	A	D	G	G	D	D
		A	G	E	A	A	A	G	P	A	T	S	Q
		R	H	R	P	P	P	L	R	V	M	Y	G
CDR3		I	F	L	L	L	L	L	G	V	I	Y	T
		I	G	G	G	G	G	G	A	V	V	D	G
		T	P	S	P	P	P	Y	T	P	V	F	W
		P	F	Y	L	L	L	F	L	A	G	W	Y
		C	D	F	D	D	D	D	Y	A	T	S	G
		M	Y	D	Y	Y	Y	Y	Y	M	L	G	G
		D	W	Y	W	W	W	W	Y	D	D	R	F
		V	G	W	G	G	G	G	Y	V	Y	G	D
		W	Q	G	Q	Q	Q	Q	Y	W	W	G	F
		G	G	Q	G	G	G	G	G	G	G	M	W
		Q	T	G	T	T	T	T	M	Q	Q	D	G
		G	L	T	L	L	L	L	D	G	G	V	Q
		T	V	L	V	V	V	V	V	T	T	W	G
		T	T	V	T	T	T	T	W	T	L	G	T
		V	V	T	V	V	V	V	G	V	V	Q	L
		T	S	V	S	S	S	S	Q	T	T	G	V
		V	S	S	S	S	S	S	G	V	V	T	T
		S	A	S	A	A	A	A	T	S	S	T	V
		S	S	A	S	S	S	S	T	S	S	V	S
		A	T	S	T	T	T	T	V	A	A	T	S
		S	K	T	K	K	K	K	T	S	S	V	A
		T	G	K	G	G	G	G	V	T	T	S	S
		K	P	G	P	P	P	P	S	K	K	S	T
		G	S	P	S	S	S	S	S	G	G	A	K
		P	V	S	V	V	V	V	A	P	P	S	G
		S	F	V	F	F	F	F	S	S	S	T	P
		V	P	F	P	P	P	P	T	V	V	K	S
		F	L	P	L	L	L	L	K	F	F	G	V
		P	A	L	A	A	A	A	G	P	P	P	F
		L	P	A	P	P	P	P	P	L	L	S	P
		A	C	P	C	C	C	C	S	A	A	V	L
		P	S	C	S	S	S	S	V	P	P	F	A
		C	R	S	R	R	R	R	F	C	C	P	P
		S	S	R	S	S	S	S	P	S	S	L	C
		R	T	S	T	T	T	T	L	R	R	A	S
		S	S	T	S	S	S	S	A	S	S	P	R
		T	E	S	E	E	E	E	P	T	T	C	S
		S	S	E	S	S	S	S	C	S	S	S	T
		E	T	S	T	T	T	T	S	E	E	R	S

Figure 2

CDR	DP50	3.1.1	4.1.1	4.8.1	4.10.2	4.13.1	4.14.3	6.1.1	11.2.1	11.6.1	11.7.1	12.3.1.1	12.9.1.1
		S	A	T	A	A	A	A	R	S	S	S	E
		T	A	A	A	A	A	A	S	T	T	T	S
		A	L	A	L	L	L	L	T	A	A	S	T
		A	G	L	G	G	G	G	S	A	A	E	A
		L	C	C	C	C	C	C	E	L	L	S	A
		G	L	C	L	L	L	L	S	G	G	T	L
		C	V	L	V	V	V		T	C	C	A	G
		L	K	V	K	K	K		A	L	L	A	C
		V	D	K	D	D	D		A	V	V	L	L
		K	Y	D	Y	Y	Y		L	K	K	G	V
		D	F	Y	F	F	F		G	D	D	C	K
		Y	P	F	P	P	P		C	Y	Y	L	D
		F	E	P	E	E	E		L	F	F	V	Y
		P	P	E	P	P	P		V	P	P	K	F
		E	V	P	V	V	V		K	E	E	D	P
		P	T	V	T	T	T		D	P	P	Y	E
		V	V	T	V	V	V		Y	V		F	P
		T	S	V	S	S	S		F	T		P	V
		V	W	S	W	W	W		P	V		E	T
		S	N	W	N	N	N		E			P	V
		W	S	N	S	S	S		P			V	S
		N	G	S	G	G	G		V			T	W
		S	A	G	A	A	A		T			V	N
		G	L	A	L	L	L		V			S	S
		A	T	L	T	T	T		S			W	G
		L	S	T	S	S	S		W			N	A
		T	G	S	G		G		N			S	L
		S	V	G	V		V		S			G	T
		G	H	V	H		H		G			A	S
		V	T	H	T		T		A			L	G
		H	F	T	F		F		L			T	V
		T	P	F	P		P		T			S	H
		F	A	P	A		A		S			G	T
		P	V	A	V		V		G			V	F
		A	L	V	L		L		V			H	
		V	Q		Q		Q		H			T	
		L										F	
		Q										P	
												A	
												V	

FIGURE 3**DP-65 or 4-31 gene product**

VSGGSISSGGYYWQHPGKGLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSTAAADTAVYYCAR
 CDR1 CDR2

2.1.3 Heavy Chain Protein

SGPGLVKPSQILSLTCTVSGGSISSGGHHYTWQWIRQHPGKGLEWIGYIYYIGNTYYNPSLKSRVTISVDTSKNQFSLKLSSTAAADTAVYYCAR
 CDR1 CDR2
 DSGDYYGIDVWGQGTITVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 CDR3

FIGURE 4**A27 Gene Product**

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGSSP
 CDR1 CDR2 CDR3

4.1.1 Kappa Chain Protein

QSPGTLSPGERATLSCRASQSISSFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGTSPT
 CDR1 CDR2 CDR3
 FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK

4.8.1 Kappa Chain Protein

QSPGTLSPGERATLSCRITs00VSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGLSPFT
 CDR1 CDR2 CDR3
 FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.14.3 Kappa Chain Protein

GTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGRSPFT
 CDR1 CDR2 CDR3
 FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

6.1.1 Kappa Chain Protein

QSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLIYGSSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGLSPFT
 CDR1 CDR2 CDR3
 FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.10.2 Kappa Chain Protein

SPGTLSPGERATLSCRASQSISSNFLAWYQQKPGQAPRLLIYRPSSRATGIPDSFSGSGGTDFLTISRLEPEDFALYYCOOYGLSPFT
 CDR1 CDR2 CDR3
 FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.13.1 Kappa Chain Protein

QSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCOOYGRSPFT
 CDR1 CDR2 CDR3
 FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKGG

Figure 5**012 Gene Product**

DIQMTQSPSSLSASVGDRVTITCRASQISSYLNWYQQKPKAPKLLIYAASSLOS^{CDR2}GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQOQSYSTP^{CDR3}

3.1.1 Kappa Chain Protein

QSPSSLSASVGDRVTITCRASQINITYLIWYQQKPKAPN^{CDR1}FLISATISLOS^{CDR2}GVPSRFRGSGSGTNFTLTINSLHPEDFATYYCQOQSYSTP^{CDR3}

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASV^{CDR1}VCLLN^{CDR2}NYFPREAKVQWKVDNALQSG

11.2.1 Kappa Chain Protein

PSSLSASVGDRVTITCRASQINISYLDWYQQKPKAPKLLIYAASSLOS^{CDR1}GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQOQSYSTP^{CDR3}

FGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV^{CDR2}VCLLN^{CDR3}NYFPREAKV

11.6.1 Kappa Chain Protein

TQSPSSLSASVGDRVTITCRASQINISRYLNWYQQKPKAPKFLIYVASILOS^{CDR1}GVPSGFASGSGPDFTLTISSLQPEDFATYYCQOQSYSTP^{CDR3}

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASV^{CDR2}VCLLN

11.7.1 Kappa Chain Protein

TQSPSSLSASVGDRVTITCRASQINCNYLNWYQQKPKAPRVLIYAASSLO^{CDR1}GGVPSRFGSGSGIDCTLTISSSLQPEDFATYYCQOQSYSTP^{CDR3}

FGPGTRVDIERTVAAPSVFIFPPSDEQLKSGTASV^{CDR2}VCLLN^{CDR3}NYFPREAKVQWKVDNAY

Figure 6

A10/A26 Gene Product

EIVLTQSPDFQSVTPKEKVTITTCRASOSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGTDFTLTINSLEAEDAATYYCHQSSSLPQ
 CDR1 CDR2 CDR3

2.1.3 Kappa Chain Protein

SPDFQSVTPKEKVTITTCRASOSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGTDFTLTINSLEAEDAATYYCHQSSSLP
 LT
 FGGGTKVEIKRTVAAPSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE
 CDR1 CDR2 CDR3

Figure 7**A17 Gene Product**

DVVMTQSPVTLGQPASISCRSSOSLYYSDGNTYLNWFQQRPGQSPRRLLYKVSNRDSGVDPDRFSGSGGTDTLTKISRVEAEDVGYYVYCMQGTHTWP
 CDR1 CDR2 CDR3

12.3.1 Kappa Chain Protein

PLSLPVTGQPASISCRSSOSLYYSDGNTYLNWFQQRPGQSPRRLLYKVSNWDSGVDPDRFSGSGGTDTLTKISRVEAEDVGYYVYCMQGSHPPT
 CDR1 CDR2 CDR3

FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

Figure 8

A3/A19 Gene Product

DIVMTQSP¹LSLPVTPGEPASISCRSSQSL²HSNGYN³YLDWY⁴LQKPGQSPQ⁵LLIY⁶LGSN⁷RASGV⁸PD⁹RFSGSG¹⁰SGTD¹¹FTLK¹²ISRVEA¹³EDVGV¹⁴YY¹⁵CMQAL¹⁶QTP¹⁷
 CDR1 CDR2 CDR3

12.9.1 Kappa Chain Protein

PGEPASISCRSSQSL²HSNGYN³YLDWY⁴LQKPGQSPQ⁵LLIY⁶LGSN⁷RASGV⁸PD⁹RFSGSG¹⁰SGTD¹¹FTLK¹²SRVEA¹³EDVGV¹⁴YY¹⁵CMQAL¹⁶QTP¹⁷
 FGGG¹⁸TKV¹⁹EIK²⁰RTVA²¹APSV²²FI²³PPS²⁴DEQL²⁵KS²⁶GTAS²⁷VV²⁸CL²⁹LN³⁰NY³¹PR³²
 CDR1 CDR2 CDR3

Amino-terminal amino acid sequence analysis

Hybridoma	Light chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH ₂ -DIQMTQSPSSLSASVGDRV	26,119
CT4.1.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,917
CT4.8.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,617
CT4.9.1	NH ₂ -DIQMTQSPSSVSASVGDRV	23,702
CT4.10.2	NH ₂ -TGEFVLTQSPGTLSPGER (60 %) NH ₂ -EFVLTQSPGTLSPGERAT (40 %)	24,101
CT4.14.3	NH ₂ -EIVLTQSPGTLSPGERAT	23,770
CT4.13.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,802
CT6.1.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,747

Hybridoma	Heavy chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~80%) NH ₂ -PEVQF... (minor sequence~20%)	51,813
CT4.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~65%) NH ₂ -PEVQFNWYVD... (minor sequence~35%)	51,502
CT4.8.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPG(R)SL... (major sequence~60%) NH ₂ -PEVQFNWY... (minor sequence~40%)	51,597
CT4.9.1	NH ₂ -EVQLLES GGGLVQPGGSLRL (free amino terminus)	51,437
CT4.10.2	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~60%) NH ₂ -PEVQFNWYVD... (minor sequence~40%)	51,502
CT4.14.3	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSL(R)(L)(S) (major sequence~65%) NH ₂ -PEVQFNWYV... (minor sequence~35%)	51,293
CT4.13.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~75%) NH ₂ -PEVQFN... (minor sequence~25%)	51,305
CT6.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVEPGRSLRLS * (major sequence~65%) NH ₂ -PEVQFNWYVD... (minor sequence~35%)	51,476

* This heavy chain sequence is similar to the other blocked heavy chain sequences except for a unique Gln->Glu change at position 13.

ABGENIX - CONFIDENTIAL

Figure 10

Figure 10A

antibody	Conc. (mg/ml) (Ec1.58)		IEF	SDS-PAGE		SEC	reported MALDI		n-term. seq. (k)*	
	reported	observed		(+) b-me	(-) b-me		Hc	Lc	reported	observed
CT 3.1.1	1.1	1.57	smear	50 & 28 kDa	6 bands	139,400	51,813	26,119	DIQMTQSP	DIQMTQSP
CT 4.1.1	1.54	1.65	smear	50 & 24 kDa	6 bands	79,900	51,502	23,917	EIVLTQSP	EIVLTQSP
CT 4.8.1	1.52	1.54	4 bands	50 & 24 kDa	6 bands	110,300	51,597	23,617	EIVLTQSP	EIVLTQSP
CT 4.10.2	1.29	1.77	4 bands	50 & 25 kDa	6 bands	107,200	51,502	24,101	**	***
CT 4.14.3	1.75	1.65	smear	50 & 24 kDa	6 bands	82,800	51,293	23,770	EIVLTQSP	EIVLTQSP
CT 6.1.1	1.36	1.3	4 bands	50 & 24 kDa	6 bands	101,100	51,476	23,747	EIVLTQSP	EIVLTQSP
* all heavy chains n-terminally blocked (not sequenced in-house)										
** mixed sequence reported: TGEFVLQSP (60) & EFVLQSP (40)										
*** mixed sequence observed TGEFVLQSP (60) & EFVLQSP (40)										

1OD_{280nm} = 0.633 mg/ml
Ec=1.58

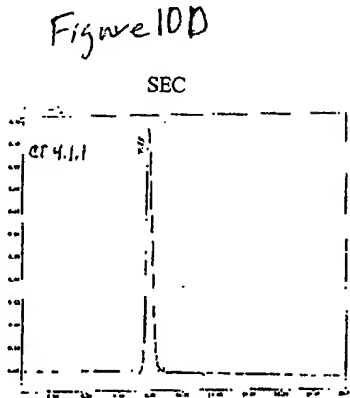
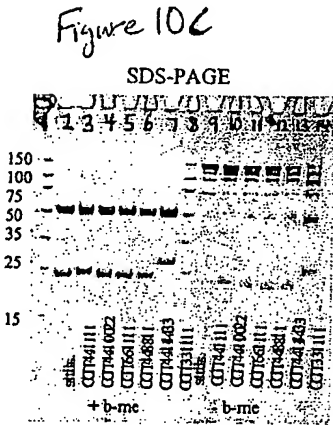
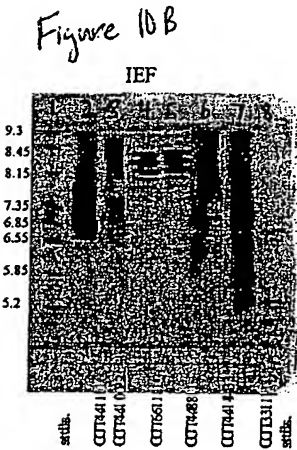


Figure 11

Expression of B7.1 and B7.2 on Raji Cells

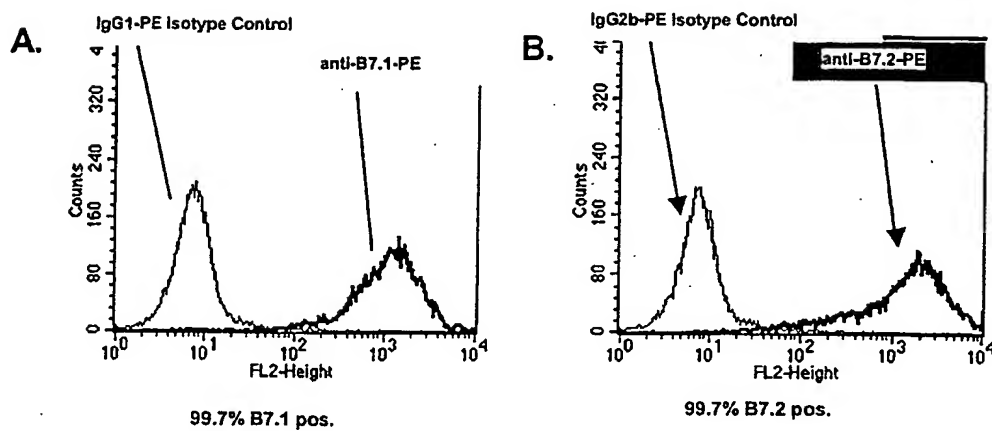


Figure 12

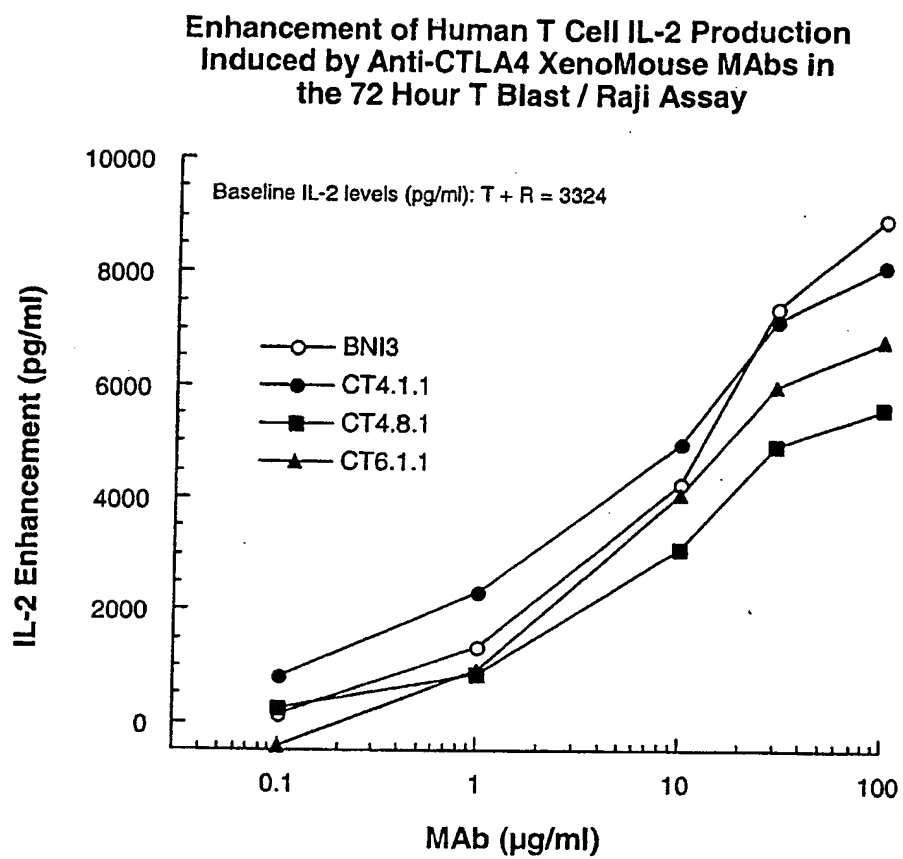


Figure 13

**Enhancement of Human T Cell IFN- γ Production
Induced by Anti-CTLA4 XenoMouse MAbs in
the 72 Hour T Blast / Raji Assay**

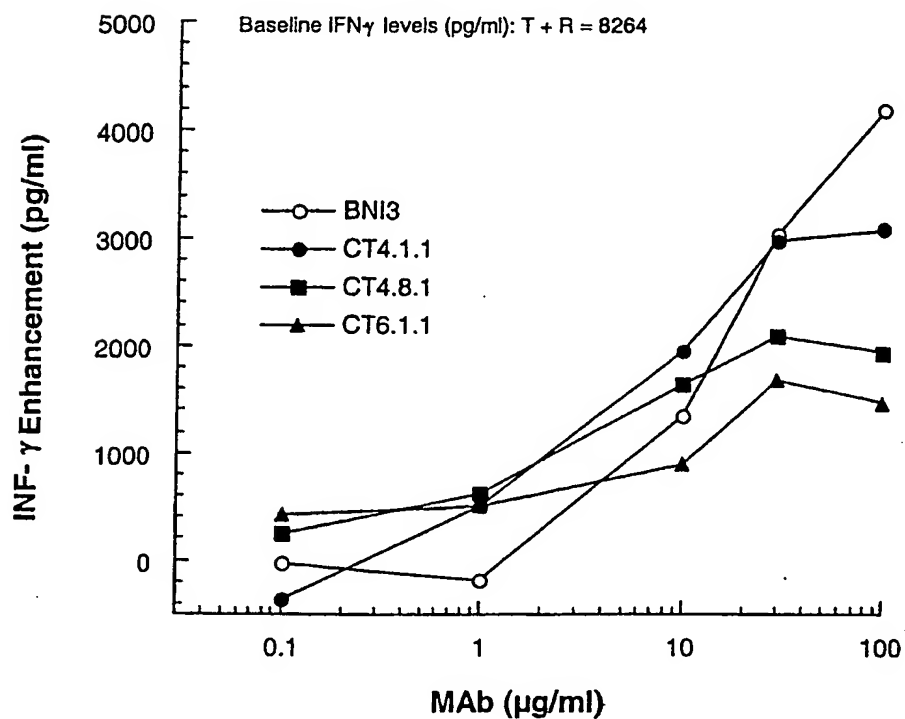


Figure 14

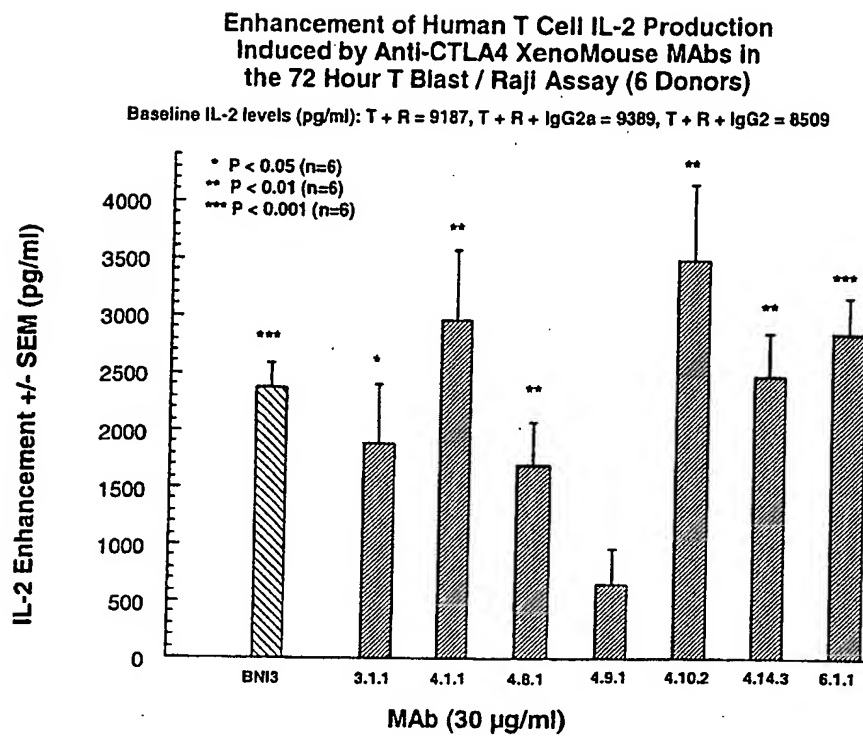


Figure 15

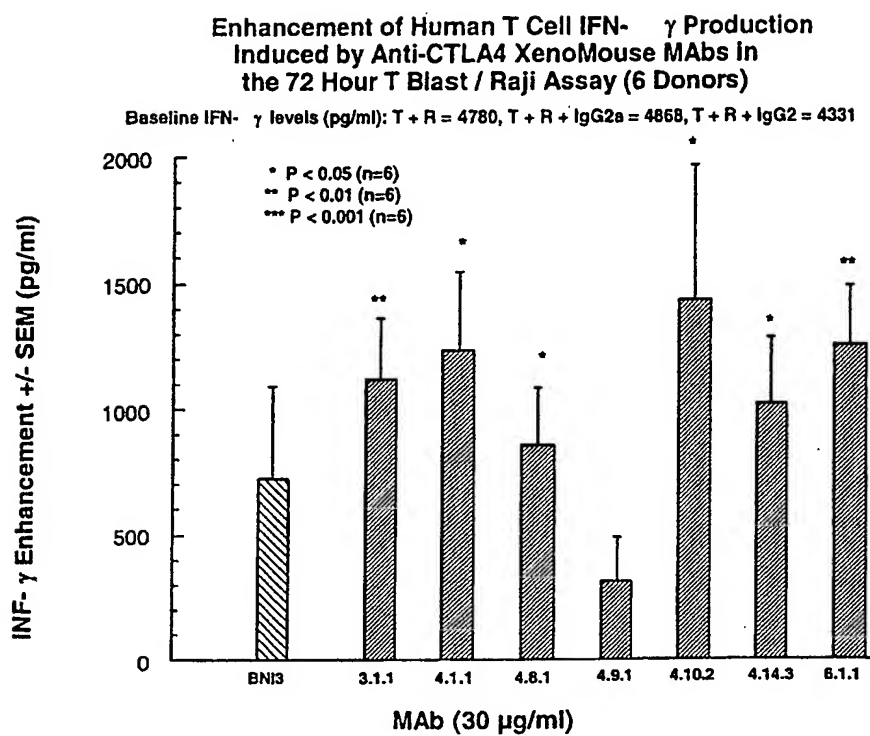


Figure 1b

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAb CT4.1.1 (30 μ g/ml) Binding to Human PBMC Stimulated with SEA (100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)

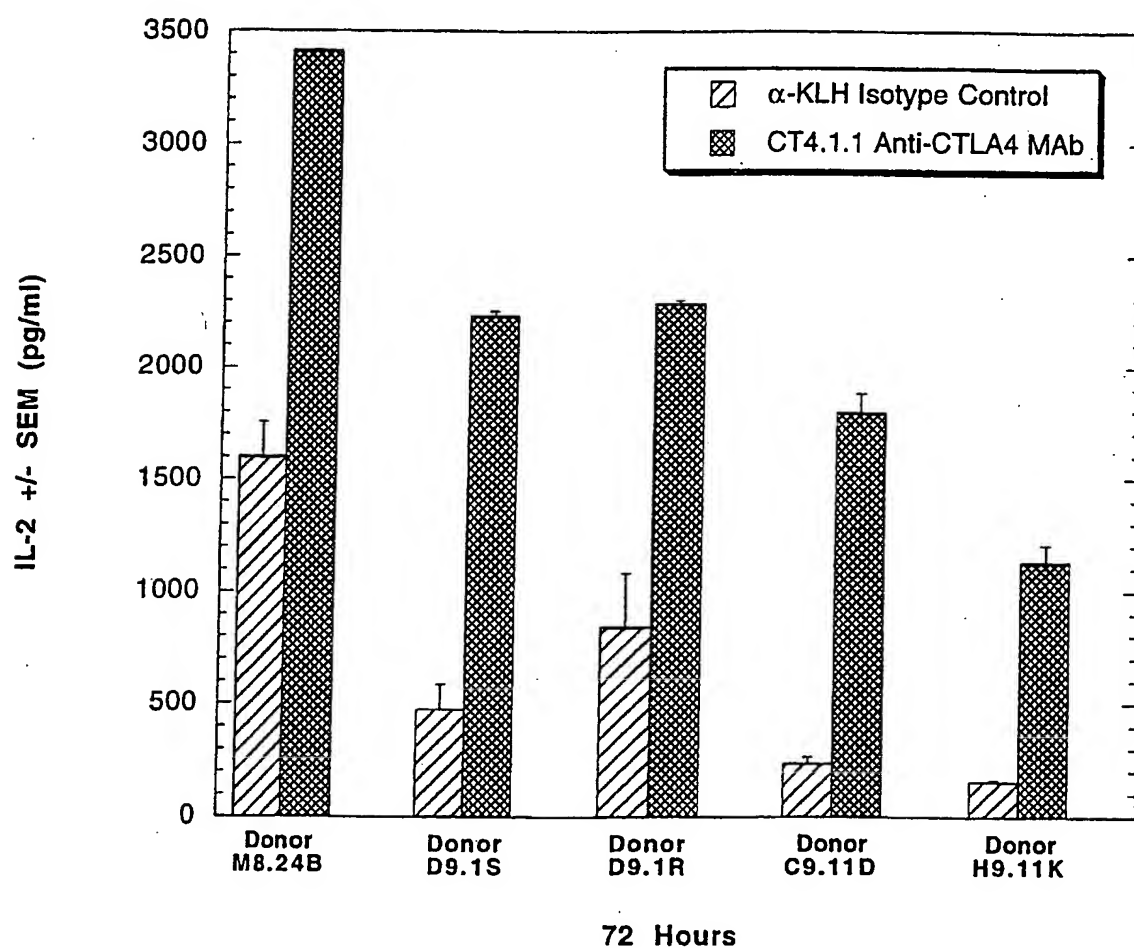


Figure 17

**Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs
(30 μ g/ml) in Human Whole Blood Stimulated with SEA
(100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)**

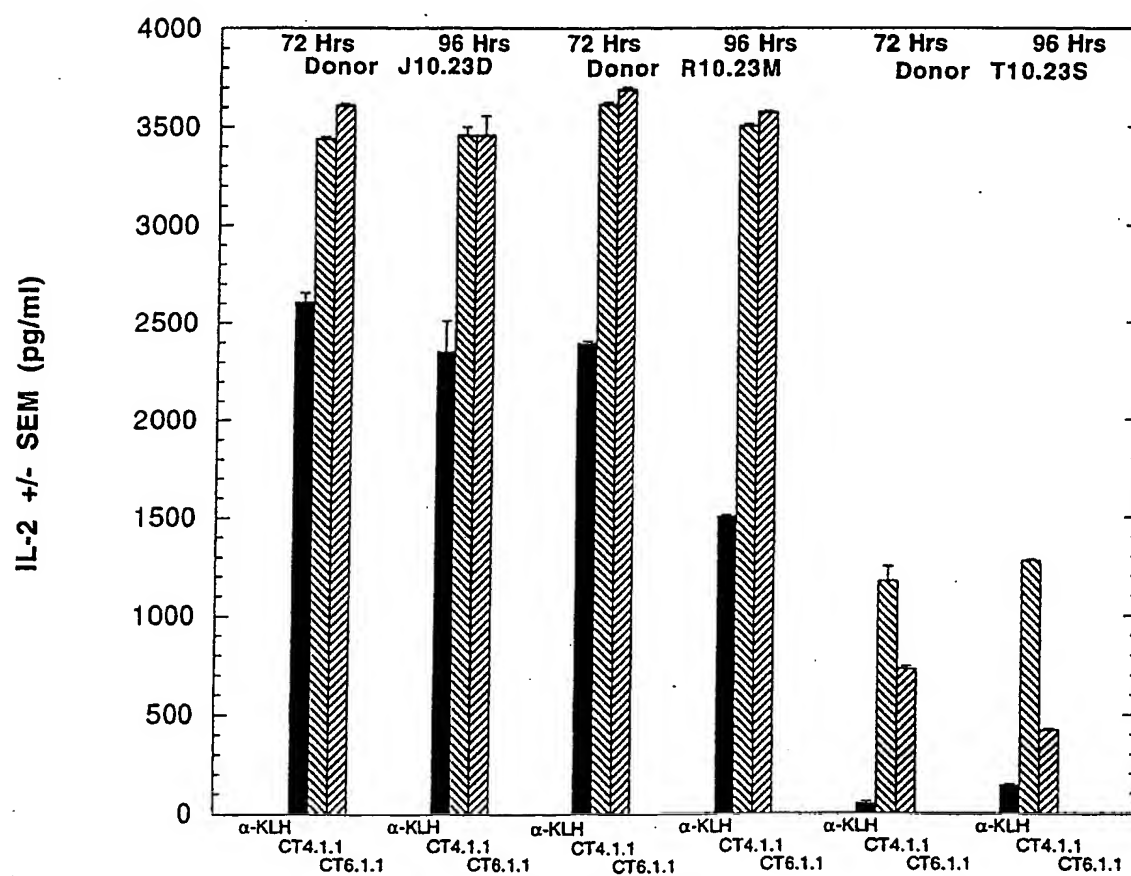
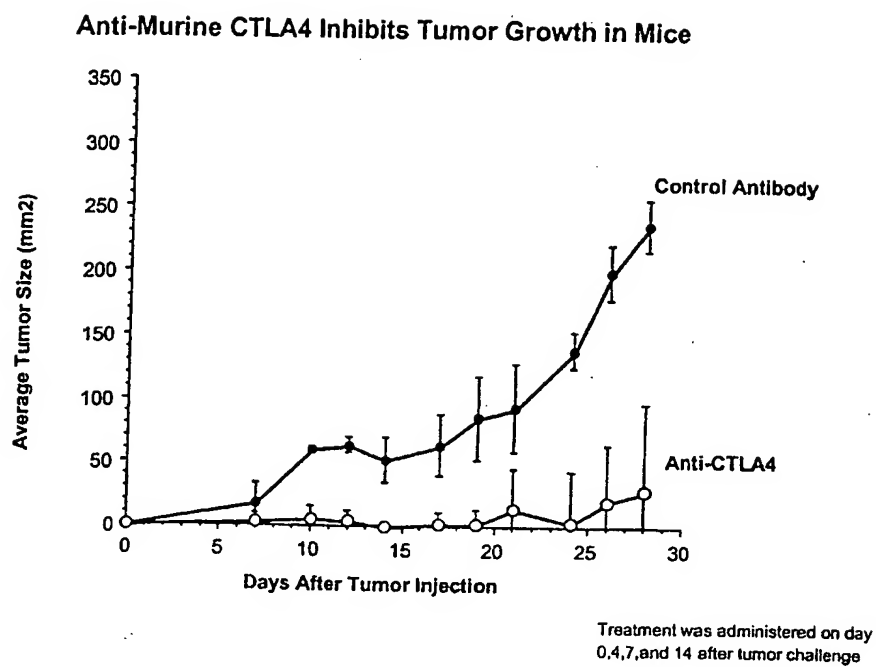
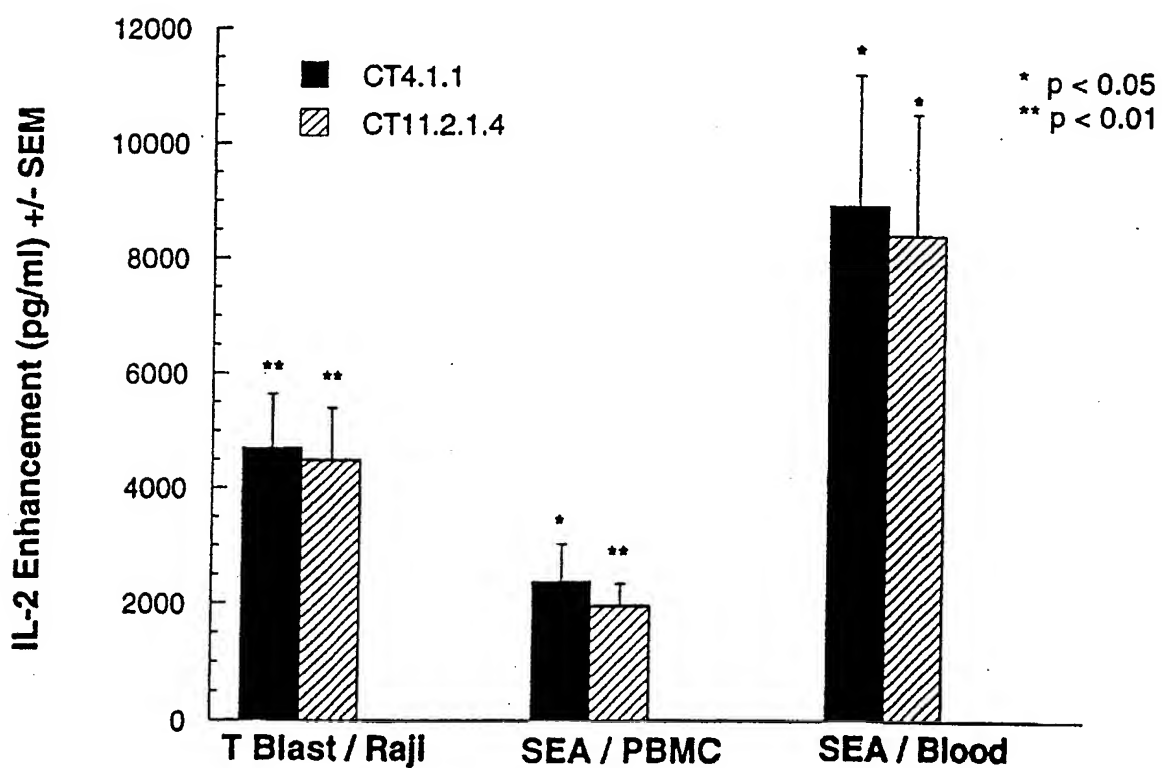


Figure 18



**Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs
(30 μ g/ml) in the 72 Hour T Blast / Raji and
Superantigen Assays (6 Donors)**



IL-2 4.1.1 vs 11.2.1

Enhancement of Human T Cell IL-2 Production Induced by Anti-CTLA4 MAbs in the 72 Hour T Blast / Raji Assay

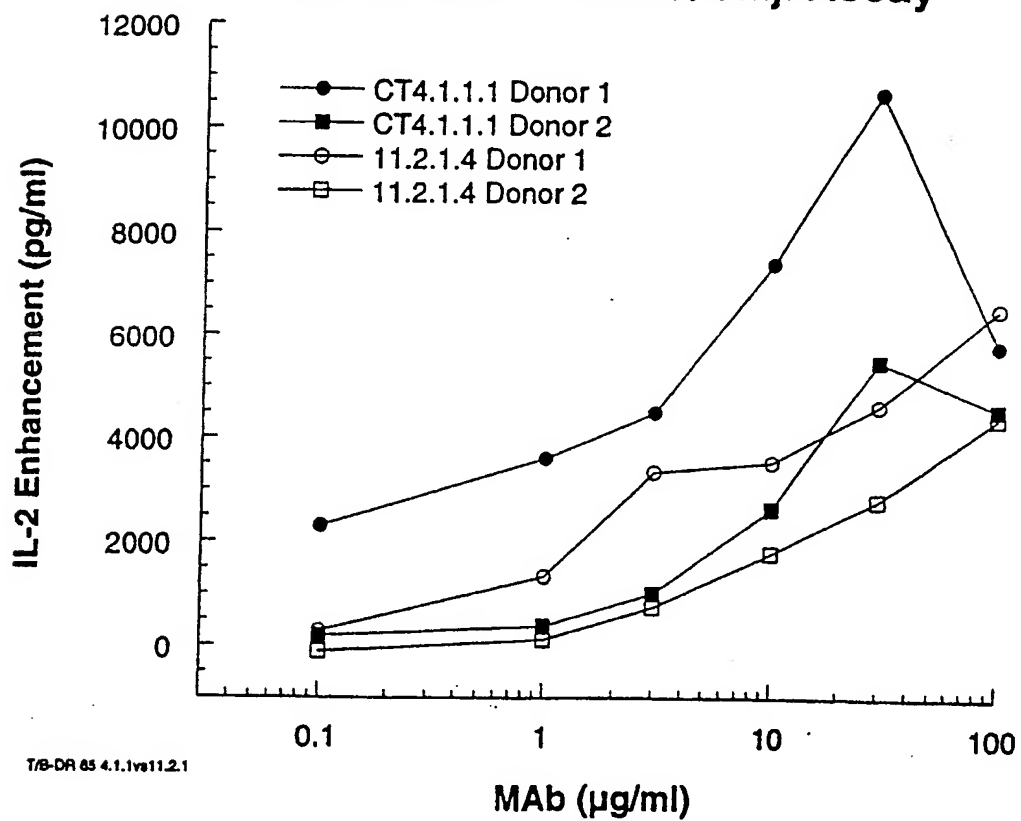


Figure 21

**Enhancement of IL-2 Production Induced by Anti-CTLA4
MAbs in Whole Blood Stimulated with
Superantigen (100 ng/ml)**

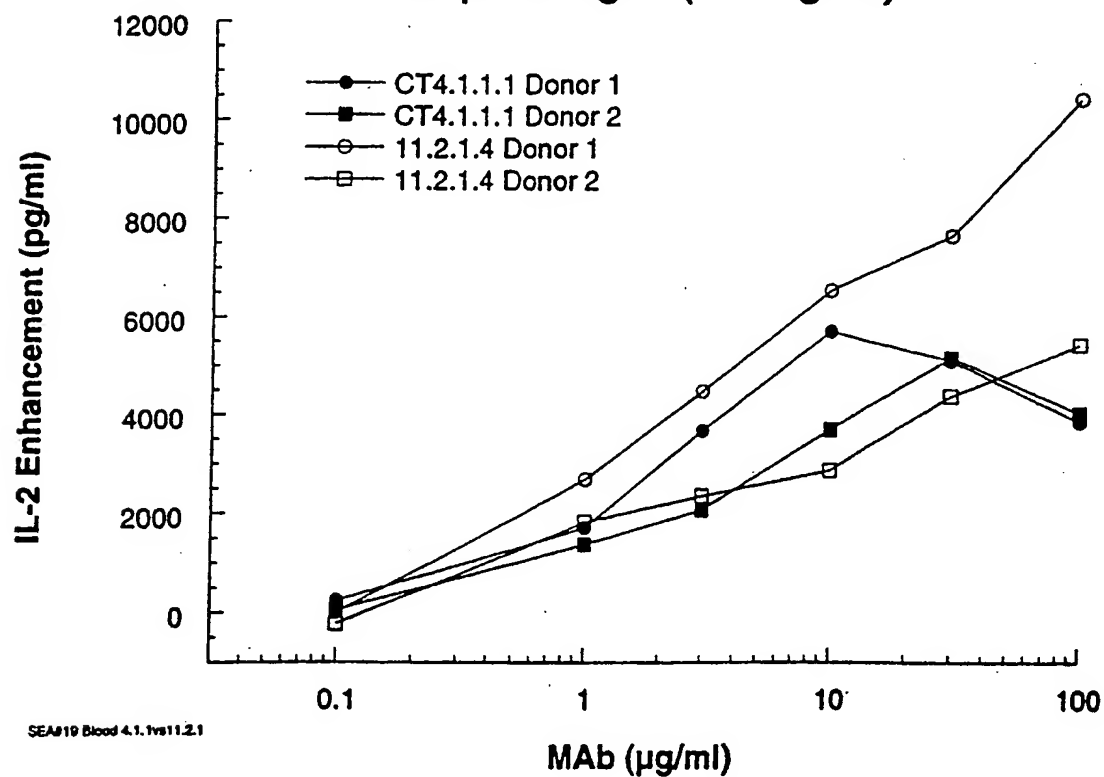


FIGURE 22

- Signal peptides shown in bold and large text
- Open reading frame for genomic clone underlined
- Mutations introduced to make deglycosylated Ab (N294Q) double underlined and large text

Fig 22(a) 4.1.1 IgG2 Heavy Chain cDNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
 CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC
 CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
 AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC
 GATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
 AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT
 CGGTCTTTTGGTACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCT
 CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
 GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
 GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
 CTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
 TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
 CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGCGAGTGCCAC
 CGTGCCCGAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA
 CCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGTGGT
 GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
 TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG
 TTCCGTGTGGTCAGCGTCTCACCCTTGTGCACCAGGACTGGCTGAACGGCAA
 GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCATCGAGAAAA
 CCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCC
 CCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAA
 AGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
 AGAACAATAACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
 CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
 TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:53)

Fig. 22(b) 4.1.1 IgG2 Heavy Chain
Genomic DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
 CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC
 CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
 AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC

GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACCTT
CGGTCTTTTGTACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCCTCAGCTA
GCACCAAGGGCCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
CTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGGTGAGAGGCCAGCTCAGGGAGGGAGGG
TGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCACCCCGGCTGTGC
AGCCCCAGCCCAGGGCAGCAAGGCAGGCCCCATCTGTCTCCTCACCCGGAGGC
CTCTGCCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTTCCACCA
GGCTCCAGGCAGGCACAGGCTGGGTGCCCTACCCAGGCCCTTCACACACAG
GGGCAGGTGCTTGGCTCAGACCTGCCAAAAGCCATATCCGGGAGGACCCTGCC
CCTGACCTAAGCCGACCCCAAAGGCCAAACTGTCCACTCCCTCAGCTCGGACA
CCTTCTCTCCTCCAGATCCGAGTAACCTCCAATCTTCTCTCTGCAGAGCGCA
AATGTTGTGTGAGTGCCCAACCGTGCCCAAGGTAAGCCAGCCCAGGCCTCGCCC
TCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGC
CCCAGCTGGGTGCTGACACGTCCACCTCCATCTCTTCTCCTCAGCACCCACCTGTG
GCAGGACCGTCAGTCTTCTCTTCCCCCCTCAAGGACACCCCTCATGAT
CTCCCGGACCCCTGAGGTACGTGCGTGGTGGTGGACGTGAGCCACGAAGACC
CCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG
ACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCT
CACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCT
CCAACAAAGGCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAGGT
GGGACCCGCGGGGTATGAGGGCCACATGGACAGAGGCCGGCTCGGCCACCCT
CTGCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGCAGCCCCGA
GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA
GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGG
AGTGGGAGAGCAATGGGCAGCCGGAGAACTACAAGACCACACCTCCCATG
CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAG
CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ
 ID NO: 54)

Fig. 22(c) 4.1.1 IgG2 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSRLRLSCVASGFTFSS
 HGMHWVRQAPGKLEWVAVIWDGRNKYYADSVKGRFTISRDNKNTLFLQMN
 SLRAEDTAVYYCARGGHFGPFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS
 ESTAALGLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTP
 SSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPPK
 PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
 FRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPVYTLTP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFF
 LYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK (SEQ ID
 NO: 63)

Fig. 22(d) 4.1.1 IgG2 Heavy Chain cDNA
N294Q

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTCACCTTCAGTAGC
CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT
CGGTCCTTTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCCTCAGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCAG
CTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCAG
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGCGAGTGCCAC
CGTGCCCGAGCACCACTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCCCAAA
CCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACCGTGCGTGGTGGT
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCCAAGCACG
TTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCAGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAA
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAACCTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:55)

Fig. 22(e) 4.1.1 IgG2 Heavy Chain
Protein N294Q

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCVASGFTFSS
HGMHWVRQAPGKLEWVAVIWDGRNKYYADSVKGRFTISRDN SKNTLFLQMN
SLRAEDTAVYYCARGGHFGPFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS
ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSNFGTQTYTCNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVAGPSVFLFPPK
PKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQST
FRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL P
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDS DGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:64)

Fig. 22(f) 4.1.1 Kappa Chain DNA

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC
CAGATACCACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTT
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTATTAGC
AGCAGCTTCTTAGCCTGGTACCAGCAGAGACCTGGCCAGGCTCCCAGGCTCCT
CATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAAGTGGCA
GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGAT
TTTGCAGTGTATTACTGTCAGCAGTATGGTACCTCACCCTGGACGTTCCGGCCA
AGGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCT
TCCCCGCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTG
CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGC
CCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAA
CACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
AAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:56)

Fig. 22(g) 4.1.1 Kappa Chain Protein

METPAQLLFLLLLWLDPDTTGEIVLTQSPGTLSSLSPGERATLSCRASQSI
SSFLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPED
FAVYYCQQYGTSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEK
HKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO:65)

Fig 22(h) 4.8.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCAGTAAC
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGTAATAAACACTATGGAGACTCCGTGAAGGGCC
GATTCACCATCTCCAGTGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGAGACT
GGGGTCTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG
CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC
TCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC
GGTGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCC
CAGCTGTCTTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG
CCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCAAAAGCC
CAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGCGAGTGCC
CACCGTGCCAGCACCACTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCA

AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGT
GGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGC
ACGTTCCGTGTGGTCAGCGTCCTCACC GTTGTGCACCAGGACTGGCTGAACGG
CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGA
AAACCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGT
CAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACAAC TACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTC
TTCTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGA
GCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:57)

Fig. 22(i) 4.8.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSRLSCTASGFTFSN
YGMHWVRQAPGKGLEWVAVIWDGNSKHYGDSVKGRFTISSDNSKNTLYLQMN
SLRAEDTAVYYCARGERLGSYFDYWGGTLVTVSSASTKGPSVFPLAPCSRST
SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSNFGTQTYTCNV DHKPSNTKVDKTV ERKCCVECP PAPPVAGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PMLDS DGSF
FLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID
NO:66)

Fig. 22(j) 4.8.1 Kappa Chain DNA

ATGGAAACCC CAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC
CAGATAACCACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTT
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGACCAGTGTTAGCAGCAGT
TACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTA
TGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCA GTGGCAGTGGGT
CTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTGCA
GTCTATTACTGT CAGCAGTATGGCATCTCACCCTTCACTTTCGGCGGAGGGAC
CAAGGTGGAGATCAAGCGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC
CATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAAT
AACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCA
ATCGGGTAACTCCCAGGAGAGTGT CACAGAGCAGGACAGCAAGGACAGCACCT
ACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAA
GTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG
CTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:58)

Fig. 22(k) 4.8.1 Kappa Chain Protein

METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSSLSPGERATLSCRTSVSSS
YLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA
VYYCQQYGISPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:67)

Fig. 22(l) 6.1.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTTCGAGC
CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTCACCTTCAGTAGT
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGCAATAAACTATGCAGACTCCGCGAAGGGCC
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGCCGGACTGCT
GGGTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
CTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCAC
CGTGCCCGAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGTGGT
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG
TTCCGTGTGGTCAGCGTCTCACC GTTGTGCACCAGGACTGGCTGAACGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAA
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAACCTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:59)

Fig. 22(m) 6.1.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVEPGRSLRLSCTASGFTFSS
YGMHWVRQAPGKLEWVAVIWDGSKHYADSAKGRFTISRDNKNTLYLQMN
SLRAEDTAVYYCARAGLLGYFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS
ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSNFGTQTYTCNVDPKPSNTKVDKTKVERKCCVECPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST

FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLTP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSGSFF
 LYSKLTVDKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
 NO: 68)

Fig. 22(n) 6.1.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC
 CAGATACCAACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTT
 TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGTAGGGCCAGTCAAAGTGTTAGC
 AGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCCCTCAT
 CTATGGTGTATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAAGTGGCAGTG
 GGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT
 GCAGTGTATTACTGTCAGCAGTATGGTATCTCACCATTCACTTTTCGGCCCTGG
 GACCAAAGTGGATATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
 CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
 AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT
 CCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA
 CCTACAGCCTCAGCAGCACCTTGACGCTGAGCAAAGCAGACTACGAGAAACAC
 AAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAA
 GAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 60)

Fig. 22(o) 6.1.1 Kappa Chain Protein

METPAQLLFLLLLWLPD TTGEIVLTQSPGTL SLSLSPGERATL SCRASQSVS
 SYLAWYQQKPGQAPRPLIYGVSSRATGIPDRFSGSGSGTDFTLTISRLEPEDF
 AVYYCQQYGISPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
 NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKH
 KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 69)

Fig. 22(p) 11.2.1 IgG2 Heavy Chain DNA:

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCCTCGTTGCTCTTTTAAGAG
 GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
 CTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGC
 TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
 AGTTATATGGTATGATGGAAGTAATAATACTATGCAGACTCCGTGAAGGGCC
 GATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC
 AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCGAGGGG
 AGCTACCCTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCA
 CGGTACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCG
 CCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAA
 GGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTCAAGGCGCTCTGACCA
 GCGGCGTGACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC

AGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTG
 CAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCA
 AATGTTGTGTGAGTGCCACCGTGCCAGCACCACCTGTGGCAGGACCGTCA
 GTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCC
 TGAGGTACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGT
 TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGG
 GAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCA
 CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCC
 TCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAA
 CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGT
 CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGT
 GGGAGAGCAATGGGCAGCCGGAGAACAATAAGACCACACCTCCCATGCTG
 GACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
 GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA
 ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
 NO:61)

**Fig. 22(q) 11.2.1 IgG2 Heavy Chain
 Protein:**

QVQLVESGGGVVQPGRLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIYW
 DGSNKYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARDPRGATLY
 YYYYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP
 EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDH
 KPSNTKVDKTVERKCCVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC
 VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWL
 NGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTC
 LVKGFYPSPDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:70)

Fig. 22(r) 11.2.1 IgG2 Kappa Chain DNA:

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCT
 GGCTCCGAGGTGCCAGATGTGACATCCAGATGACCCAGTCTCCATCCTCC
 CTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAG
 CATTAAACAGCTATTTAGATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAAC
 TCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGT
 GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGA
 AGATTTTGCAACTTACTACTGTCAACAGTATTACAGTACTCCATTCACTTTCG
 GCCCTGGGACCAAAGTGGAATCAAACGAACTGTGGCTGCACCATCTGTCTTC
 ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTG
 CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATA
 ACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAG
 GACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGA
 GAAACACAAAGTCTACGCCTGCGAAGTACCCATCAGGGCCTGAGCTCGCCCCG
 TCACAAAGAGCTTCAACAGGGGAGAGTGTTAGTGA (SEQ ID NO:62)

Fig. 22(s) 11.2.1 IgG2 Kappa Chain
Protein:

DIQMTQSPSSLSASVGDRVTITCRASQSINSYLDWYQQKPGKAPKLLIYAASS
LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYYSTPFTFGPGTKVEI
KRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC (SEQ ID NO:71)

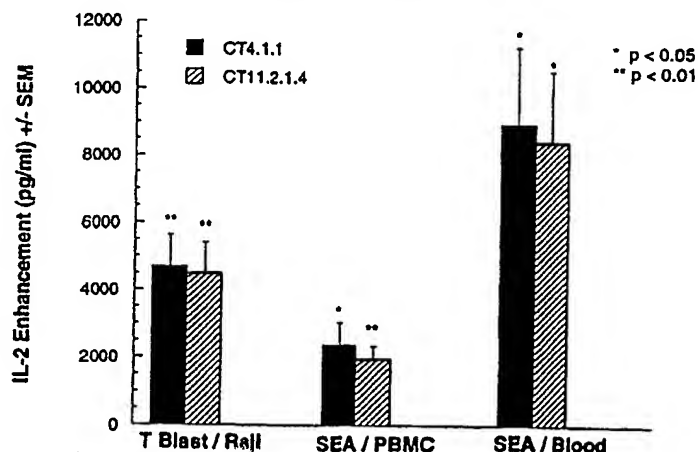


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 16/28, C12N 5/08, G01N 33/53, 33/68		A3	(11) International Publication Number: WO 00/37504
			(43) International Publication Date: 29 June 2000 (29.06.00)
(21) International Application Number: PCT/US99/30895		(74) Agent: HARE, Christopher, A.; Abgenix, Inc., 7601 Dumbarton Circle, Fremont, CA 94555 (US).	
(22) International Filing Date: 23 December 1999 (23.12.99)			
(30) Priority Data: 60/113,647 23 December 1998 (23.12.98) US		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71) Applicants (for all designated States except US): PFIZER, INC. [US/US]; Eastpoint Road, Groton, CT 06340 (US). AB-GENIX, INC. [US/US]; 7601 Dumbarton Circle, Fremont, CA 94555 (US).			
(72) Inventors; and		Published With international search report.	
(75) Inventors/Applicants (for US only): HANSON, Douglas, Charles [US/US]; 3 Acorn Drive, Niantic, CT 06357 (US). NEVEU, Mark, Joseph [US/US]; 18 Greenbriar Court, Mystic, CT 06355 (US). MUELLER, Eileen, Elliott [US/US]; 4 Butterwick Lane, Old Lyme, CT 06371 (US). HANKE, Jeffrey, Herbert [US/US]; 1 Jefferson Circle, Reading, MA 01867 (US). GILMAN, Steven, Christopher [US/US]; 118 Sill Lane, Old Lyme, CT 06371 (US). DAVIS, C., Geoffrey [US/US]; 1132 Vancouver Avenue, Burlingame, CA 94010 (US). CORVALAN, Jose, Ramon [CL/US]; 125 Williams Lane, Foster City, CA 94010 (US).		(88) Date of publication of the international search report: 14 September 2000 (14.09.00)	

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Superantigen Assays (6 Donors)



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/30895

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/28 C12N5/08 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 42752 A (BRIGHAM AND WOMEN'S HOSPITAL) 1 October 1998 (1998-10-01) claims	1-56
A	WO 95 33770 A (REPLIGEN CORPORATION ET AL.) 14 December 1995 (1995-12-14) page 17, line 19 - line 29 claim 12	1-56
A	A. JAKOBOVITS: "The long-awaited magic bullets: therapeutic human monoclonal antibodies from transgenic mice." EXPERT OPINION ON INVESTIGATIONAL DRUGS, vol. 7, no. 4, April 1998 (1998-04), pages 607-614, XP002084509 London, GB abstract figures 1,3	1-56



Further documents are listed in the continuation of box C.



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Date of the actual completion of the international search

24 May 2000

Date of mailing of the international search report

13/06/2000

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/30895

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 33735 A (CELL GENESYS, INC.) 31 October 1996 (1996-10-31) page 14, line 19 - line 28 claim 20	1-56
A	WO 98 50433 A (ABGENIX, INC.) 12 November 1998 (1998-11-12) examples claims	1-56
A	P. MARRACK ET AL.: "The staphylococcal enterotoxins and their relatives." SCIENCE, vol. 248, no. 4956, 11 May 1990 (1990-05-11), pages 705-711, XP002138564 New York, NY, USA the whole document	86-104

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. Appl. Application No

PCT/US 99/30895

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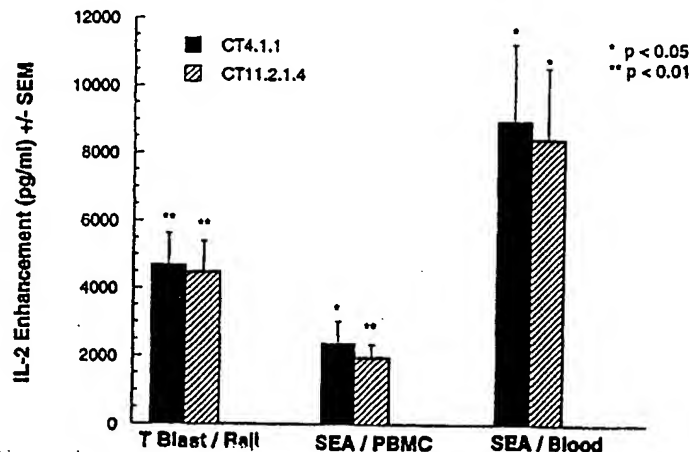


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 16/28, C12N 5/08, G01N 33/53, 33/68	A3	(11) International Publication Number: WO 00/37504 (43) International Publication Date: 29 June 2000 (29.06.00)
(21) International Application Number: PCT/US99/30895 (22) International Filing Date: 23 December 1999 (23.12.99) (30) Priority Data: 60/113,647 23 December 1998 (23.12.98) US (71) Applicants (for all designated States except US): PFIZER, INC. [US/US]; Easternpoint Road, Groton, CT 06340 (US). AB-GENIX, INC. [US/US]; 7601 Dumbarton Circle, Fremont, CA 94555 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HANSON, Douglas, Charles [US/US]; 3 Acorn Drive, Niantic, CT 06357 (US). NEVEU, Mark, Joseph [US/US]; 18 Greenbriar Court, Mystic, CT 06355 (US). MUELLER, Eileen, Elliott [US/US]; 4 Butterwick Lane, Old Lyme, CT 06371 (US). HANKE, Jeffrey, Herbert [US/US]; 1 Jefferson Circle, Reading, MA 01867 (US). GILMAN, Steven, Christopher [US/US]; 118 Sill Lane, Old Lyme, CT 06371 (US). DAVIS, C., Geoffrey [US/US]; 1132 Vancouver Avenue, Burlingame, CA 94010 (US). CORVALAN, Jose, Ramon [CL/US]; 125 Williams Lane, Foster City, CA 94010 (US).		(74) Agent: HARE, Christopher, A.; Abgenix, Inc., 7601 Dumbarton Circle, Fremont, CA 94555 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. (88) Date of publication of the international search report: 14 September 2000 (14.09.00)

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In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Also provided is a cell culture system for assaying T cell stimulation.

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HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

BACKGROUND OF THE INVENTION

5 1. *Cross-Reference to Related Applications*

The present application claims priority to U.S. Provisional Patent Application, Serial No. 60/113,647, filed December 23, 1998, the disclosure of which is hereby incorporated in its entirety herein.

10

 2. *Summary of the Invention*

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4
15 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are
20 antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.

 3. *Background of the Technology*

25 Regulation of immune response in patients would provide a desirable treatment of many human diseases that could lead to a specificity of action that is rarely found through the use of conventional drugs. Both up-regulation and down-regulation of responses of the immune system would be possible. The roles of T cells and B cells have been extensively studied and characterized in
30 connection with the regulation of immune response. From these studies, the role of T cells appear, in many cases, to be particularly important in disease prevention and treatment.

T cells possess very complex systems for controlling their interactions. Interactions between T cells utilize numerous receptors and soluble factors for the process. Thus, what effect any particular signal may have on the immune response generally varies and depends on the particular factors, receptors and counter-receptors that are involved in the pathway. The pathways for down-regulating responses are as important as those required for activation. Thymic education leading to T-cell tolerance is one mechanism for preventing an immune response to a particular antigen. Other mechanisms, such as secretion of suppressive cytokines, are also known.

Activation of T cells requires not only stimulation through the antigen receptor (T cell receptor (TCR)), but additional signaling through co-stimulatory surface molecules such as CD28. The ligands for CD28 are the B7-1 (CD80) and B7-2 (CD86) proteins, which are expressed on antigen-presenting cells such as dendritic cells, activated B-cells or monocytes that interact with T-cell CD28 or CTLA-4 to deliver a costimulatory signal. The role of costimulatory signaling was studied in experimental allergic encephalomyelitis (EAE) by Perrin et al. *Immunol Res* 14:189-99 (1995). EAE is an autoimmune disorder, induced by Th1 cells directed against myelin antigens that provides an *in vivo* model for studying the role of B7-mediated costimulation in the induction of a pathological immune response. Using a soluble fusion protein ligand for the B7 receptors, as well as monoclonal antibodies specific for either CD80 or CD86, Perrin et al. demonstrated that B7 costimulation plays a prominent role in determining clinical disease outcome in EAE.

The interaction between B7 and CD28 is one of several co-stimulatory signaling pathways that appear to be sufficient to trigger the maturation and proliferation of antigen specific T-cells. Lack of co-stimulation, and the concomitant inadequacy of IL-2 production, prevent subsequent proliferation of the T cell and induce a state of non-reactivity termed "anergy". A variety of viruses and tumors may block T cell activation and proliferation, leading to insufficient activity or non-reactivity of the host's immune system to the infected or transformed cells. Among a number of possible T-cell disturbances,

anergy may be at least partly responsible for the failure of the host to clear the pathogenic or tumorigenic cells.

The use of the B7 protein to mediate anti-tumor immunity has been described in Chen et al. *Cell* 71:1093-1102 (1992) and Townsend and Allison
5 *Science* 259:368 (1993). Schwartz *Cell* 71:1065 (1992) reviews the role of CD28, CTLA-4, and B7 in IL-2 production and immunotherapy. Harding et al. *Nature* 356:607-609 (1994) demonstrates that CD28 mediated signaling co-stimulates murine T cells and prevents the induction of anergy in T cell clones. See also U.S. Patent Nos. 5,434,131, 5,770,197, and 5,773,253, and
10 International Patent Application Nos. WO 93/00431, WO 95/01994, WO 95/03408, WO 95/24217, and WO 95/33770.

From the foregoing, it was clear that T-cells required two types of signals from the antigen presenting cell (APC) for activation and subsequent differentiation to effector function. First, there is an antigen specific signal
15 generated by interactions between the TCR on the T-cell and MHC molecules presenting peptides on the APC. Second, there is an antigen-independent signal that is mediated by the interaction of CD28 with members of the B7 family (B7-1 (CD80) or B7-2 (CD86)). Exactly where CTLA-4 fit into the milieu of immune responsiveness was initially evasive. Murine CTLA-4 was first
20 identified and cloned by Brunet et al. *Nature* 328:267-270 (1987), as part of a quest for molecules that are preferentially expressed on cytotoxic T lymphocytes. Human CTLA-4 was identified and cloned shortly thereafter by Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988). The murine and human CTLA-4 molecules possess approximately 76% overall sequence homology and
25 approach complete sequence identity in their cytoplasmic domains (Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988)). CTLA-4 is a member of the immunoglobulin (Ig) superfamily of proteins. The Ig superfamily is a group of proteins that share key structural features of either a variable (V) or constant (C) domain of Ig molecules. Members of the Ig superfamily include, but are not
30 limited to, the immunoglobulins themselves, major histocompatibility complex (MHC) class molecules (i.e., MHC class I and II), and TCR molecules.

In 1991, Linsley et al. *J. Exp. Med.* 174:561-569 (1991), proposed that CTLA-4 was a second receptor for B7. Similarly, Harper et al. *J Immunol* 147:1037-44 (1991) demonstrated that the CTLA-4 and CD28 molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *See also* Balzano et al. *Int J Cancer Suppl* 7:28-32 (1992). Further evidence of this role arose through functional studies. For example, Lenschow et al. *Science* 257:789-792 (1992) demonstrated that CTLA-4-Ig induced long term survival of pancreatic islet grafts. Freeman et al. *Science* 262:907-909 (1993) examined the role of CTLA-4 in B7 deficient mice. Examination of the ligands for CTLA-4 are described in Lenschow et al. *P.N.A.S.* 90:11054-11058 (1993). Linsley et al. *Science* 257:792-795 (1992) describes immunosuppression *in vivo* by a soluble form of CTLA-4. Linsley et al. *J Exp Med* 176:1595-604 (1992) prepared antibodies that bound CTLA-4 and that were not cross-reactive with CD28 and concluded that CTLA-4 is coexpressed with CD28 on activated T lymphocytes and cooperatively regulates T cell adhesion and activation by B7. Kuchroo et al. *Cell* 80:707-18 (1995) demonstrated that the B7-1 and B7-2 costimulatory molecules differentially activated the Th1/Th2 developmental pathways. Yi-qun et al. *Int Immunol* 8:37-44 (1996) demonstrated that there are differential requirements for co-stimulatory signals from B7 family members by resting versus recently activated memory T cells towards soluble recall antigens. *See also* de Boer et al. *Eur J Immunol* 23:3120-5 (1993).

Several groups proposed alternative or distinct receptor/ligand interactions for CTLA-4 as compared to CD28 and even proposed a third B-7 complex that was recognized by a BB1 antibody. *See, for example*, Hathcock et al. *Science* 262:905-7 (1993), Freeman et al. *Science* 262:907-9 (1993), Freeman et al. *J Exp Med* 178:2185-92 (1993), Lenschow et al. *Proc Natl Acad Sci U S A* 90:11054-8 (1993), Razi-Wolf et al. *Proc Natl Acad Sci U S A* 90:11182-6 (1993), and Boussiotis et al. *Proc Natl Acad Sci U S A* 90:11059-63 (1993). *But, see*, Freeman et al. *J Immunol* 161:2708-15 (1998) who discuss finding that BB1 antibody binds a molecule that is identical to the cell surface form of CD74 and, therefore, the BB1 mAb binds to a protein distinct from B7-

1, and this epitope is also present on the B7-1 protein. Thus, this observation required the field to reconsider studies using BB1 mAb in the analysis of CD80 expression and function.

Beginning in 1993 and culminating in 1995, investigators began to
5 further delineate the role of CTLA-4 in T-cell stimulation. First, through the use of monoclonal antibodies against CTLA-4, Walunas et al. *Immunity* 1:405-13 (1994) provided evidence that CTLA-4 can function as a negative regulator of T cell activation. Thereafter, Waterhouse et al. *Science* 270:985-988 (1995) demonstrated that mice deficient for CTLA-4 accumulated T cell blasts with up-
10 regulated activation markers in their lymph nodes and spleens. The blast cells also infiltrated liver, heart, lung, and pancreas tissue, and amounts of serum immunoglobulin were elevated and their T cells proliferated spontaneously and strongly when stimulated through the T cell receptor, however, they were sensitive to cell death induced by cross-linking of the Fas receptor and by
15 gamma irradiation. Waterhouse et al. concluded that CTLA-4 acts as a negative regulator of T cell activation and is vital for the control of lymphocyte homeostasis. In a comment in the same issue, Allison and Krummel *Science* 270:932-933 (1995), discussed the work of Waterhouse et al. as demonstrative that CTLA-4 acts to down regulate T-cell responsiveness or has an inhibitory
20 signaling role in T-cell activation and development. Tivol et al. *Immunity* 3:541-7 (1995) also generated CTLA-4-deficient mice and demonstrated that such mice rapidly develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis. They concluded that CTLA-4 plays a key role in down-regulating
25 T cell activation and maintaining immunologic homeostasis. Also, Krummel and Allison *J Exp Med* 182:459-65 (1995) further clarified that CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. They generated an antibody to CTLA-4 and investigated the effects of its binding to CTLA-4 in a system using highly purified T cells. In their report, they showed
30 that the presence of low levels of B7-2 on freshly explanted T cells can partially inhibit T cell proliferation, and this inhibition was mediated by interactions with CTLA-4. Cross-linking of CTLA-4 together with the TCR and CD28 strongly

inhibits proliferation and IL-2 secretion by T cells. Finally, the results showed that CD28 and CTLA-4 deliver opposing signals that appear to be integrated by the T cell in determining the response to antigen. Thus, they concluded that the outcome of T cell antigen receptor stimulation is regulated by CD28
5 costimulatory signals, as well as inhibitory signals derived from CTLA-4. *See also* Krummel et al. *Int Immunol* 8:519-23 (1996) and U.S. Patent No. 5,811,097 and International Patent Application No. WO 97/20574.

A variety of additional experiments have been conducted further elucidating the above function of CTLA-4. For example, Walunas et al. *J Exp*
10 *Med* 183:2541-50 (1996), through the use of anti-CTLA-4 antibodies, suggested that CTLA-4 signaling does not regulate cell survival or responsiveness to IL-2, but does inhibit CD28-dependent IL-2 production. Also, Perrin et al. *J Immunol* 157:1333-6 (1996), demonstrated that anti-CTLA-4 antibodies in experimental allergic encephalomyelitis (EAE), exacerbated the disease and enhanced
15 mortality. Disease exacerbation was associated with enhanced production of the encephalitogenic cytokines TNF-alpha, IFN-gamma and IL-2. Thus, they concluded that CTLA-4 regulates the intensity of the autoimmune response in EAE, attenuating inflammatory cytokine production and clinical disease manifestations. *See also* Hurwitz et al. *J Neuroimmunol* 73:57-62 (1997) and
20 Cepero et al. *J Exp Med* 188:199-204 (1998) (an anti-CTLA-4 hairpin ribozyme that specifically abrogates CTLA-4 expression after gene transfer into a murine T-cell model).

In addition, Blair et al. *J Immunol* 160:12-5 (1998) assessed the functional effects of a panel of CTLA-4 monoclonal antibodies (mAbs) on
25 resting human CD4+ T cells. Their results demonstrated that some CTLA-4 mAbs could inhibit proliferative responses of resting CD4+ cells and cell cycle transition from G0 to G1. The inhibitory effects of CTLA-4 were evident within 4 h, at a time when cell surface CTLA-4 expression remained undetectable. Other CTLA-4 mAbs, however, had no detectable inhibitory effects, indicating
30 that binding of mAbs to CTLA-4 alone was not sufficient to mediate down-regulation of T cell responses. Interestingly, while IL-2 production was shut off, inhibitory anti-CTLA-4 mAbs permitted induction and expression of the cell

survival gene bcl-X(L). Consistent with this observation, cells remained viable and apoptosis was not detected after CTLA-4 ligation.

In connection with anergy, Perez et al. *Immunity* 6:411-7 (1997) demonstrated that the induction of T cell anergy was prevented by blocking CTLA-4 and concluded that the outcome of antigen recognition by T cells is determined by the interaction of CD28 or CTLA-4 on the T cells with B7 molecules. Also, Van Parijs et al. *J Exp Med* 186:1119-28 (1997) examined the role of interleukin 12 and costimulators in T cell anergy *in vivo* and found that through inhibiting CTLA-4 engagement during anergy induction, T cell proliferation was blocked, and full Th1 differentiation was not promoted. However, T cells exposed to tolerogenic antigen in the presence of both IL-12 and anti-CTLA-4 antibody were not anergized, and behaved identically to T cells which have encountered immunogenic antigen. These results suggested that two processes contribute to the induction of anergy *in vivo*: CTLA-4 engagement, which leads to a block in the ability of T cells to proliferate, and the absence of a prototypic inflammatory cytokine, IL-12, which prevents the differentiation of T cells into Th1 effector cells. The combination of IL-12 and anti-CTLA-4 antibody was sufficient to convert a normally tolerogenic stimulus to an immunogenic one.

In connection with infections, McCoy et al. *J Exp Med* 186:183-7 (1997) demonstrated that anti-CTLA-4 antibodies greatly enhanced and accelerated the T cell immune response to *Nippostrongylus brasiliensis*, resulting in a profound reduction in adult worm numbers and early termination of parasite egg production. See also Murphy et al. *J. Immunol.* 161:4153-4160 (1998) (*Leishmania donovani*).

In connection with cancer, Kwon et al. *PNAS USA* 94:8099-103 (1997) established a syngeneic murine prostate cancer model and examined two distinct manipulations intended to elicit an antiprostata cancer response through enhanced T cell costimulation: (i) provision of direct costimulation by prostate cancer cells transduced to express the B7.1 ligand and (ii) *in vivo* antibody-mediated blockade of T cell CTLA-4, which prevents T cell down-regulation. It was demonstrated that *in vivo* antibody-mediated blockade of CTLA-4

enhanced antiprostata cancer immune responses. Also, Yang et al. *Cancer Res* 57:4036-41 (1997) investigated whether the blockade of the CTLA-4 function leads to enhancement of antitumor T cell responses at various stages of tumor growth. Based on *in vitro* and *in vivo* results they found that CTLA-4 blockade
5 in tumor-bearing individuals enhanced the capacity to generate antitumor T-cell responses, but the expression of such an enhancing effect was restricted to early stages of tumor growth in their model. Further, Hurwitz et al. *Proc Natl Acad Sci U S A* 95:10067-71 (1998) investigated the generation of a T cell-mediated antitumor response depends on T cell receptor engagement by major
10 histocompatibility complex/antigen as well as CD28 ligation by B7. Certain tumors, such as the SM1 mammary carcinoma, were refractory to anti-CTLA-4 immunotherapy. Thus, through use of a combination of CTLA-4 blockade and a vaccine consisting of granulocyte-macrophage colony-stimulating factor-expressing SM1 cells, regression of parental SM1 tumors was observed, despite
15 the ineffectiveness of either treatment alone. This combination therapy resulted in long-lasting immunity to SM1 and depended on both CD4(+) and CD8(+) T cells. The findings suggested that CTLA-4 blockade acts at the level of a host-derived antigen-presenting cell.

In connection with diabetes, Luhder et al. *J Exp Med* 187:427-32 (1998)
20 injected an anti-CTLA-4 mAb into a TCR transgenic mouse model of diabetes at different stages of disease. They found that engagement of CTLA-4 at the time when potentially diabetogenic T cells are first activated is a pivotal event; if engagement is permitted, invasion of the islets occurs, but remains quite innocuous for months. If not, insulinitis is much more aggressive, and diabetes
25 quickly ensues.

In connection with vaccine immunization, Horspool et al. *J Immunol* 160:2706-14 (1998) found that intact anti-CTLA-4 mAb but not Fab fragments suppressed the primary humoral response to pCIA/beta gal without affecting recall responses, indicating CTLA-4 activation inhibited Ab production but not
30 T cell priming. Blockade of the ligands for CD28 and CTLA-4, CD80 (B7-1) and CD86 (B7-2), revealed distinct and nonoverlapping function. Blockade of CD80 at initial immunization completely abrogated primary and secondary Ab

responses, whereas blockade of CD86 suppressed primary but not secondary responses. Simultaneous blockade of CD80 + CD86 was less effective at suppressing Ab responses than either alone. Enhancement of costimulation via coinjection of B7-expressing plasmids augmented CTL responses but not Ab
5 responses, and without evidence of Th1 to Th2 skewing. These findings suggest complex and distinct roles for CD28, CTLA-4, CD80, and CD86 in T cell costimulation following nucleic acid vaccination.

In connection with allograft rejection, Markees et al. *J Clin Invest* 101:2446-55 (1998) found in a mouse model of skin allograft rejection that
10 acceptance initially depended on the presence of IFN-gamma, CTLA-4, and CD4(+) T cells. Addition of anti-CTLA-4 or anti-IFN-gamma mAb to the protocol was associated with prompt graft rejection, whereas anti-IL-4 mAb had no effect.

In connection with the role of CTLA-4 in relation to CD28, Fallarino et al. *J Exp Med* 188:205-10 (1998) generated TCR transgenic/recombinase
15 activating gene 2-deficient/CD28-wild-type or CD28-deficient mice which were immunized with an antigen-expressing tumor. Primed T cells from both types of mice produced cytokines and proliferated in response to stimulator cells lacking B7 expression. However, whereas the response of CD28+/+ T cells was augmented by costimulation with B7-1, the response of the CD28-/- T cells was
20 strongly inhibited. This inhibition was reversed by monoclonal antibody against B7-1 or CTLA-4. Thus, CTLA-4 can potently inhibit T cell activation in the absence of CD28, indicating that antagonism of a TCR-mediated signal is sufficient to explain the inhibitory effect of CTLA-4. Also, Lin et al. *J Exp Med* 25 188:199-204 (1998) studied rejection of heart allografts in CD28-deficient mice. H-2(q) hearts were transplanted into allogeneic wild-type or CD28-deficient mice (H-2(b)). Graft rejection was delayed in CD28-deficient compared with wild-type mice. Treatment of wild-type recipients with CTLA-4-immunoglobulin (Ig), or with anti-B7-1 plus anti-B7-2 mAbs significantly
30 prolonged allograft survival. In contrast, treatment of CD28-deficient mice with CTLA-4-Ig, anti-B7-1 plus anti-B7-2 mAbs, or a blocking anti-CTLA-4 mAb induced acceleration of allograft rejection. This increased rate of graft rejection

was associated with more severe mononuclear cell infiltration and enhanced levels of IFN-gamma and IL-6 transcripts in donor hearts of untreated wild-type and CTLA-4-Ig- or anti-CTLA-4 mAb-treated CD28-deficient mice. Thus, the negative regulatory role of CTLA-4 extends beyond its potential ability to prevent CD28 activation through ligand competition. Even in the absence of CD28, CTLA-4 plays an inhibitory role in the regulation of allograft rejection.

Also, further characterization of the expression of CTLA-4 has been investigated. For example, Alegre et al. *J Immunol* 157:4762-70 (1996) proposed that surface CTLA-4 is rapidly internalized, which may explain the low levels of expression generally detected on the cell surface. They concluded that both CD28 and IL-2 play important roles in the up-regulation of CTLA-4 expression. In addition, the cell surface accumulation of CTLA-4 appeared to be primarily regulated by its rapid endocytosis. Also, Castan et al. *Immunology* 90:265-71 (1997) based on *in situ* immunohistological analyses of the expression of CTLA-4, suggested that germinal center T cells, which were CTLA-4 positive, could be important to immune regulation.

Accordingly, in view of the broad and pivotal role that CTLA-4 appears to possess in immune responsiveness, it would be desirable to generate antibodies to CTLA-4 that can be utilized effectively in immunotherapy. Moreover, it would be desirable to generate antibodies against CTLA-4 that can be utilized in chronic diseases in which repeat administrations of the antibodies are required.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

Figure 1 provides a series of nucleotide and an amino acid sequences of heavy chain and kappa light chain immunoglobulin molecules in accordance with the invention: 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M).

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1,

4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "0s".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17

amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

5 Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as
10 underlined.

Figure 9 provides a summary of N-terminal amino acid sequences generated through direct protein sequencing of the heavy and light chains of the antibodies.

Figure 10 provides certain additional characterizing information about
15 certain of the antibodies in accordance with the invention. In Figure 10A, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. Data related to concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, liquid chromatography/mass spectroscopy (LCMS), mass spectroscopy (MALDI), light chain N-terminal sequences is provided.
20 Additional detailed information related to IEF is provided in Figure 10B; related to SDS-PAGE is provided in 10C; and SEC of the 4.1.1 antibody in 10D.

Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs.

Figure 12 shows the concentration dependent enhancement of IL-2
25 production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1).

Figure 13 shows the concentration dependent enhancement of IFN- γ production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1)(same donor T cells).

30 Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 15 shows the mean enhancement of IFN- γ production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 16 shows the enhancement of IL-2 production in hPBMC from 5 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 hours after stimulation with SEA.

Figure 17 shows the enhancement of IL-2 production in whole blood from 3 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 and 96 hours after stimulation with SEA.

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model.

Figure 19 shows enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji and Superantigen (whole blood and peripheral blood mononuclear cells from 6 donors) assays.

Figure 20 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji assay.

Figure 21 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour Superantigen whole blood assay stimulated with 100 ng/ml superantigen.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains: full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c)), full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e)), 4.1.1 light chain (cDNA 22(f) and amino acid 22(g)), full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i)), 4.8.1 light chain (cDNA 22(j) and amino acid 22(k)), full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m)), 6.1.1 light chain (cDNA 22(n) and amino acid 22(o)), full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)), and 11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided an antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V_H3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or framework sequences shown in Figure 2. In a preferred embodiment, the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70. In another preferred embodiment, the antibody further comprises a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

In accordance with a second aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:1 and a light chain variable amino acid sequence comprising SEQ ID NO:14.

In accordance with a third aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:2 and a light chain variable amino acid sequence comprising SEQ ID NO:15.

In accordance with a fourth aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:4 and a light chain variable amino acid sequence comprising SEQ ID NO:17.

In accordance with a fifth aspect of the present invention, there is provided an isolated human monoclonal antibody that is capable of binding to CTLA-4. In a preferred embodiment, antibody is capable of competing for binding with CTLA-4 with an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian species comprises mouse, rat, and rabbit and more preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2

production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a sixth aspect of the present invention, there is
5 provided a humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In a preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian
10 species comprises mouse, rat, and rabbit and preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and
15 preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the
20 antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN-
25 γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen
30 assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a seventh aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2. In a preferred embodiment, the antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

In accordance with an eighth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties: a binding affinity for CTLA-4 of about 10^{-9} or greater; inhibits binding between CTLA-4 and B7-1 with an IC₅₀ of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC₅₀ of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a ninth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties: a binding affinity for CTLA-4 of about 10^{-9} or greater; inhibits binding between CTLA-4 and B7-1 with an IC₅₀ of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC₅₀ of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a tenth aspect of the present invention, there is provided a cell culture system for assaying T cell stimulation, comprising a culture of human T cell blasts co-cultured with a Raji cell line. In a preferred embodiment, the T cell blasts are washed prior to culture with the Raji cell line.

In accordance with an eleventh aspect of the present invention, there is provided an assay for measuring T cell stimulation, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with an agent; and measuring cytokine production by the culture.

5 In accordance with an twelfth aspect of the present invention, there is provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with the moiety; and assessing cytokine production by the culture.

10 In accordance with a thirteenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

In accordance with a fourteenth aspect of the present invention, there is
15 provided a method for screening an agent for T cell stimulatory activity, comprising: contacting the agent with a cell culture comprising human T cell blasts and a Raji cell line; and assessing cytokine production by the culture.

In each of the tenth through the fourteenth aspects of the present invention, in a preferred embodiment, the T cell blasts are washed prior to
20 culture with the Raji cell line. In another preferred embodiment, the cytokine is IL-2 or IFN- γ . In a preferred embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

In accordance with a fifteenth aspect of the present invention, there is
25 provided an assay for measuring T cell stimulation, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; contacting the culture with an agent; and measuring cytokine production by the cell population.

In accordance with a sixteenth aspect of the present invention, there is
30 provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus

enterotoxin A; contacting the culture with the moiety; and assessing cytokine production by the cell population.

In accordance with a seventeenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising
5 contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.

In accordance with an eighteenth aspect of the present invention, there is provided a method for screening an agent for T cell stimulatory activity,
10 comprising: contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and assessing cytokine production by the cell population.

In each of the fifteenth through the eighteenth aspects of the present invention, in a preferred embodiment, the cytokine is IL-2. In another preferred
15 embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20

In accordance with the present invention, there are provided fully human monoclonal antibodies against human CTLA-4. Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly sequences corresponding to a contiguous heavy and light
25 chain sequences from FR1 and CDR1 through CDR3 and FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

30

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated
5 polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

The term "isolated protein" referred to herein means a protein of cDNA,
10 recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

15

The term "polypeptide" as used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain
20 immunoglobulin molecules and the human kappa light chain immunoglobulin molecules represented in Figure 1, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs
25 thereof.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism
30 (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding
5 sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding
10 sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control
15 sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

20 The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

25 The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12,
30 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant.

Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes
5 deoxyribonucleotides and ribonucleotides. The term "modified nucleotides"
referred to herein includes nucleotides with modified or substituted sugar
groups and the like. The term "oligonucleotide linkages" referred to herein
includes oligonucleotides linkages such as phosphorothioate,
phosphorodithioate, phosphoroselenoate, phosphorodiselenoate,
10 phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See
e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem.*
Soc. 106:6077 (1984); Stein et al. *Nucl. Acids Res.* 16:3209 (1988); Zon et al.
Anti-Cancer Drug Design 6:539 (1991); Zon et al. *Oligonucleotides and*
Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford
15 University Press, Oxford England (1991)); Stec et al. U.S. Patent No.
5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the
disclosures of which are hereby incorporated by reference. An oligonucleotide
can include a label for detection, if desired.

20 The term "selectively hybridize" referred to herein means to detectably
and specifically bind. Polynucleotides, oligonucleotides and fragments thereof
in accordance with the invention selectively hybridize to nucleic acid strands
under hybridization and wash conditions that minimize appreciable amounts of
detectable binding to nonspecific nucleic acids. High stringency conditions can
25 be used to achieve selective hybridization conditions as known in the art and
discussed herein. Generally, the nucleic acid sequence homology between the
polynucleotides, oligonucleotides, and fragments of the invention and a nucleic
acid sequence of interest will be at least 80%, and more typically with
preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%.
30 Two amino acid sequences are homologous if there is a partial or complete
identity between their sequences. For example, 85% homology means that 85%
of the amino acids are identical when the two sequences are aligned for

maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M.O., in *Atlas of Protein Sequence and Structure*, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1)

comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both

sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the

amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains.

5 Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are

10 aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or

15 a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide

20 derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid

25 sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. *Science* 253:164

30 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and

5 (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the

10 polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence).

15 Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each

20 incorporated herein by reference.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the

25 naturally-occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides

30 which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to CTLA-4, under suitable

binding conditions, (2) ability to block CTLA-4 binding with its receptors, or (3) ability to inhibit CTLA-4 expressing cell growth *in vitro* or *in vivo*. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence.

5 Analog typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

Peptide analogs are commonly used in the pharmaceutical industry as

10 non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics". Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p.392 (1985); and Evans et al. *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often

15 developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such

20 as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH₂NH--, --CH₂S--, --CH₂-CH₂--, --CH=CH--(cis and trans), --COCH₂--, --CH(OH)CH₂--, and --CH₂SO--, by methods well known in the art. Systematic substitution of one or

25 more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal

30 cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', F(ab')₂, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60% or 80%, and more usually greater than about 85% (as measured in an *in vitro* competitive binding assay).

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ and most preferably $\leq 10 \text{ nM}$.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the

following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, 5 predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

10 The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San 15 Francisco (1985)), incorporated herein by reference).

The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a 20 carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any 25 other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more 30 preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot

be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term patient includes human and veterinary subjects.

5

Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions

of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody
5 having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies"
10 (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).
15 Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

20

Human Antibodies and Humanization of Antibodies

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The
25 presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, it has been postulated that one can develop humanized antibodies or generate fully human antibodies through the introduction of
30 human antibody function into a rodent so that the rodent would produce antibodies having fully human sequences.

Human Antibodies

The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance

to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and
5 selected.

This general strategy was demonstrated in connection with our generation of the first XenoMouse™ strains as published in 1994. *See* Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and
10 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes.
15 This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might
20 recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light
25 chain loci, respectively, to produce XenoMouse™ mice. *See* Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

30 Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30,

1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 5 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998). *See also* European Patent No., EP 0 463 151 B1, grant published 10 June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

15 In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a μ constant region, and a second constant region 20 (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Patent No. 5,545,807 to Surani et al. and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, and 5,814,318 each to Lonberg and Kay, U.S. Patent No. 5,591,669 to Krimpenfort and Berns, U.S. Patent Nos. 5,612,205, 25 5,721,367, 5,789,215 to Berns et al., and U.S. Patent No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. Patent Application Serial Nos. 07/574,748, filed August 29, 1990, 07/575,962, filed August 31, 1990, 07/810,279, filed December 17, 1991, 07/853,408, filed March 18, 1992, 07/904,068, filed June 23, 1992, 07/990,860, filed December 16, 1992, 30 08/053,131, filed April 26, 1993, 08/096,762, filed July 22, 1993, 08/155,301, filed November 18, 1993, 08/161,739, filed December 3, 1993, 08/165,699, filed December 10, 1993, 08/209,741, filed March 9, 1994, the disclosures of

which are hereby incorporated by reference. *See also* European Patent No. 0 546 073 B1, International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of which are hereby incorporated by reference in their entirety. *See further* Taylor et al., 1992, Chen et al., 1993, Tuailon et al., 1993, Choi et al., 1993, Lonberg et al., (1994), Taylor et al., (1994), and Tuailon et al., (1995), Fishwild et al., (1996), the disclosures of which are hereby incorporated by reference in their entirety.

10 The inventors of Surani et al., cited above and assigned to the Medical Research Counsel (the "MRC"), produced a transgenic mouse possessing an Ig locus through use of the minilocus approach. The inventors on the GenPharm International work, cited above, Lonberg and Kay, following the lead of the present inventors, proposed inactivation of the endogenous mouse Ig locus
15 coupled with substantial duplication of the Surani et al. work.

 An advantage of the minilocus approach is the rapidity with which constructs including portions of the Ig locus can be generated and introduced into animals. Commensurately, however, a significant disadvantage of the minilocus approach is that, in theory, insufficient diversity is introduced through
20 the inclusion of small numbers of V, D, and J genes. Indeed, the published work appears to support this concern. B-cell development and antibody production of animals produced through use of the minilocus approach appear stunted. Therefore, research surrounding the present invention has consistently been directed towards the introduction of large portions of the Ig locus in order
25 to achieve greater diversity and in an effort to reconstitute the immune repertoire of the animals.

 Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is
30 expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody.

Thus, it would be desirable to provide fully human antibodies against CTLA-4 in order to vitiate concerns and/or effects of HAMA or HACA response.

Humanization and Display Technologies

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As was discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. To a degree, this can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. *See e.g.*, Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12:125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (*see* WO 92/02190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al. *P.N.A.S.* 84:3439 (1987) and *J.Immunol.* 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat et al. (1991) *Sequences of Proteins of Immunological Interest*, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG2, IgG3 and IgG4. Particularly preferred isotypes for antibodies of the invention are IgG2 and IgG4. Either of the human light chain constant regions, kappa or

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lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

Antibody fragments, such as Fv, F(ab')₂ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

In one approach, consensus sequences encoding the heavy and light chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, e.g. SV-40 early promoter, (Okayama et al. *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al. *P.N.A.S.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al. *Cell* 41:885 (1985)); native Ig promoters, etc.

Further, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to

additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, *supra.*, Hanes and Plutchau *PNAS USA* 94:4937-4942 (1997) (ribosomal display), Parmley and Smith *Gene* 73:305-318 (1988) (phage display), Scott *TIBS* 17:241-245 (1992), Cwirla et al. *PNAS USA* 87:6378-6382 (1990), Russel et al. *Nucl. Acids Research* 21:1081-1085 (1993), Hoganboom et al. *Immunol. Reviews* 130:43-68 (1992), Chiswell and McCafferty *TIBTECH* 10:80-84 (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

Using these techniques, antibodies can be generated to CTLA-4 expressing cells, CTLA-4 itself, forms of CTLA-4, epitopes or peptides thereof, and expression libraries thereto (*see e.g.* U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described above.

Additional Criteria for Antibody Therapeutics

As will be appreciated, it is generally not desirable to kill CTLA-4 expressing cells. Rather, one generally desires to simply inhibit CTLA-4 binding with its ligands to mitigate T cell down regulation. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. Those isotypes that do not include, without limitation, human IgG2 and human IgG4.

It will be appreciated that antibodies that are generated need not initially possess a particular desired isotype but, rather, the antibody as generated can

possess any isotype and the antibody can be isotype switched thereafter using conventional techniques that are well known in the art. Such techniques include the use of direct recombinant techniques (*see e.g.*, U.S. Patent No. 4,816,397), cell-cell fusion techniques (*see e.g.*, U.S. Patent Application No. 08/730,639, filed October 11, 1996), among others.

In the cell-cell fusion technique, a myeloma or other cell line is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line is prepared that possesses the light chain. Such cells can, thereafter, be fused and a cell line expressing an intact antibody can be isolated.

By way of example, the majority of the CTLA-4 antibodies discussed herein are human anti-CTLA-4 IgG2 antibody. Since such antibodies possess desired binding to the CTLA-4 molecule, any one of such antibodies can be readily isotype switched to generate a human IgG4 isotype, for example, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity).

Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain additional "functional" attributes that are desired through isotype switching.

Design and Generation of Other Therapeutics

In accordance with the present invention and based on the activity of the antibodies that are produced and characterized herein with respect to CTLA-4, the design of other therapeutic modalities including other antibodies, other antagonists, or chemical moieties other than antibodies is facilitated. Such modalities include, without limitation, antibodies having similar binding activity or functionality, advanced antibody therapeutics, such as bispecific antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules. Furthermore, as discussed above, the effector function of the

antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses.

In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to
5 sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels, for example.

In connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies one with a specificity to CTLA-4 and another to a second molecule that are conjugated together, (ii) a single antibody
10 that has one chain specific to CTLA-4 and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to CTLA-4 and the other molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) *see e.g.*, Fanger et al. *Immunol Methods* 4:72-81 (1994) and Wright and Harris, *supra.* and in
15 connection with (iii) *see e.g.*, Traunecker et al. *Int. J. Cancer (Suppl.)* 7:51-52 (1992).

In addition, "Kappabodies" (Ill et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" *Protein Eng* 10:949-57 (1997)), "Minibodies" (Martin et al.
20 "The affinity-selection of a minibody polypeptide inhibitor of human interleukin-6" *EMBO J* 13:5303-9 (1994)), "Diabodies" (Holliger et al. "Diabodies": small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)), or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells"
25 *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)) may also be prepared.

In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. *See e.g.*,
30 Vitetta *Immunol Today* 14:252 (1993). *See also* U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known

in the art. See e.g., Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). See also U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902. Each of immunotoxins and radiolabeled molecules
5 would be likely to kill cells expressing CTLA-4, and particularly those cells in which the antibodies of the invention are effective.

In connection with the generation of therapeutic peptides, through the utilization of structural information related to CTLA-4 and antibodies thereto, such as the antibodies of the invention (as discussed below in connection with
10 small molecules) or screening of peptide libraries, therapeutic peptides can be generated that are directed against CTLA-4. Design and screening of peptide therapeutics is discussed in connection with Houghten et al. *Biotechniques* 13:412-421 (1992), Houghten *PNAS USA* 82:5131-5135 (1985), Pinalla et al. *Biotechniques* 13:901-905 (1992), Blake and Litzi-Davis *BioConjugate Chem.*
15 3:510-513 (1992). Immunotoxins and radiolabeled molecules can also be prepared, and in a similar manner, in connection with peptidic moieties as discussed above in connection with antibodies.

Important information related to the binding of an antibody to an antigen can be gleaned through phage display experimentation. Such experiments are
20 generally accomplished through panning a phage library expressing random peptides for binding with the antibodies of the invention to determine if peptides can be isolated that bind. If successful, certain epitope information can be gleaned from the peptides that bind.

In general, phage libraries expressing random peptides can be purchased
25 from New England Biolabs (7-mer and 12-mer libraries, Ph.D.-7 Peptide 7-mer Library Kit and Ph.D.-12 Peptide 12-mer Library Kit, respectively) based on a bacteriophage M13 system. The 7-mer library represents a diversity of approximately 2.0×10^9 independent clones, which represents most, if not all, of the $20^7 = 1.28 \times 10^9$ possible 7-mer sequences. The 12-mer library contains
30 approximately 1.9×10^9 independent clones and represents only a very small sampling of the potential sequence space of $20^{12} = 4.1 \times 10^{15}$ 12-mer sequences. Each of 7-mer and 12-mer libraries are panned or screened in accordance with

the manufacturer's recommendations in which plates were coated with an antibody to capture the appropriate antibody (a goat anti-human IgG Fc for an IgG antibody for example) followed by washing. Bound phage are eluted with 0.2 M glycine-HCl, pH 2.2. After 3 rounds of selection/amplification at constant stringency (0.5% Tween), through use of DNA sequencing, one can characterize clones from the libraries that are reactive with one or more of the antibodies. Reactivity of the peptides can be determined by ELISA. For an additional discussion of epitope analysis of peptides *see also* Scott, J.K. and Smith, G.P. *Science* 249:386-390 (1990); Cwirla et al. *PNAS USA* 87:6378-6382 (1990); Felici et al. *J. Mol. Biol.* 222:301-310 (1991), and Kuwabara et al. *Nature Biotechnology* 15:74-78 (1997).

The design of gene and/or antisense therapeutics through conventional techniques is also facilitated through the present invention. Such modalities can be utilized for modulating the function of CTLA-4. In connection therewith the antibodies of the present invention facilitate design and use of functional assays related thereto. A design and strategy for antisense therapeutics is discussed in detail in International Patent Application No. WO 94/29444. Design and strategies for gene therapy are well known. However, in particular, the use of gene therapeutic techniques involving intrabodies could prove to be particularly advantageous. *See e.g.*, Chen et al. *Human Gene Therapy* 5:595-601 (1994) and Marasco *Gene Therapy* 4:11-15 (1997). General design of and considerations related to gene therapeutics is also discussed in International Patent Application No. WO 97/38137. Genetic materials encoding an antibody of the invention (such as the 4.1.1, 4.8.1, or 6.1.1, or others) may be included in a suitable expression system (whether viral, attenuated viral, non-viral, naked, or otherwise) and administered to a host for *in vivo* generation of the antibody in the host.

Small molecule therapeutics can also be envisioned in accordance with the present invention. Drugs can be designed to modulate the activity of CTLA-4 based upon the present invention. Knowledge gleaned from the structure of the CTLA-4 molecule and its interactions with other molecules in accordance with the present invention, such as the antibodies of the invention, CD28, B7,

B7-1, B7-2, and others can be utilized to rationally design additional therapeutic modalities. In this regard, rational drug design techniques such as X-ray crystallography, computer-aided (or assisted) molecular modeling (CAMP), quantitative or qualitative structure-activity relationship (QSAR), and similar technologies can be utilized to focus drug discovery efforts. Rational design allows prediction of protein or synthetic structures which can interact with the molecule or specific forms thereof which can be used to modify or modulate the activity of CTLA-4. Such structures can be synthesized chemically or expressed in biological systems. This approach has been reviewed in Capsey et al. *Genetically Engineered Human Therapeutic Drugs* (Stockton Press, NY (1988)). Indeed, the rational design of molecules (either peptides, peptidomimetics, small molecules, or the like) based upon known, or delineated, structure-activity relationships with other molecules (such as antibodies in accordance with the invention) has become generally routine. See, e.g., Fry et al. "Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor" *Proc Natl Acad Sci U S A* 95:12022-7 (1998); Hoffman et al. "A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese homology domain" *J Mol Biol* 282:195-208 (1998); Ginalski et al. "Modelling of active forms of protein kinases: p38--a case study" *Acta Biochim Pol* 44:557-64 (1997); Jouko et al. "Identification of csk tyrosine phosphorylation sites and a tyrosine residue important for kinase domain structure" *Biochem J* 322:927-35 (1997); Singh et al. "Structure-based design of a potent, selective, and irreversible inhibitor of the catalytic domain of the erbB receptor subfamily of protein tyrosine kinases" *J Med Chem* 40:1130-5 (1997); Mandel et al. "ABGEN: a knowledge-based automated approach for antibody structure modeling" *Nat Biotechnol* 14:323-8 (1996); Monfardini et al. "Rational design, analysis, and potential utility of GM-CSF antagonists" *Proc Assoc Am Physicians* 108:420-31 (1996); Furet et al. "Modelling study of protein kinase inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411" *J Comput Aided Mol Des* 9:465-72 (1995).

Further, combinatorial libraries can be designed and synthesized and used in screening programs, such as high throughput screening efforts.

Therapeutic Administration and Formulations

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It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate
10 formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as
15 LipofectinTM), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active
20 ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to excipients and carriers
25 well known to pharmaceutical chemists.

Preparation of Antibodies

Antibodies in accordance with the invention are preferably prepared
30 through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of

producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the Background, herein. In particular, 5 however, a preferred embodiment of transgenic production of mice and antibodies therefrom is disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosure of which is hereby incorporated by reference. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997), the disclosure of which is hereby incorporated by reference.

10 Through use of such technology, we have produced fully human monoclonal antibodies to a variety of antigens. Essentially, we immunize XenoMouse™ lines of mice with an antigen of interest, recover lymphatic cells (such as B-cells) from the mice that express antibodies, fuse such recovered cells with a myeloid-type cell line to prepare immortal hybridoma cell lines, and 15 such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. We utilized these techniques in accordance with the present invention for the preparation of antibodies specific to CTLA-4. Herein, we describe the production of multiple hybridoma cell lines that produce antibodies specific to CTLA-4. Further, we 20 provide a characterization of the antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

The antibodies derived from hybridoma cell lines discussed herein are designated 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 25 11.7.1, 12.3.1.1, and 12.9.1.1. Each of the antibodies produced by the aforementioned cell lines are either fully human IgG2 or IgG4 heavy chains with human kappa light chains. In general, antibodies in accordance with the invention possess very high affinities, typically possessing Kd's of from about 10^{-9} through about 10^{-11} M, when measured by either solid phase or solution 30 phase.

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines.

Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed.

Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO₀, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive CTLA-4 binding properties.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase and DHFR gene expression systems are common approaches for enhancing expression under certain

conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 5 89303964.4.

Antibodies of the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in 10 mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. *See, e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

Antibodies in accordance with the present invention have been analyzed structurally and functionally. In connection with the structures of the 15 antibodies, amino acid sequences of the heavy and kappa light chains have been predicted based on cDNA sequences obtained through RT-PCR of the hybridomas. *See* Examples 3 and 4 and Figures 1-8. N-terminal sequencing of the antibodies was also conducted in confirmation of the results discussed in Examples 3 and 4. *See* Example 5 and Figure 9. Kinetic analyses of the 20 antibodies were conducted to determine affinities. *See* Example 2. Antibodies in accordance with the invention (and particularly the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) have high affinities ($4.1.1:1.63 \times 10^{10}$ 1/M; $4.8.1:3.54 \times 10^{10}$ 1/M; and $6.1.1:7.2 \times 10^9$ 1/M). Further, antibodies were analyzed by isoelectric focusing (IEF), reducing gel electrophoresis (SDS- 25 PAGE), size exclusion chromatography, liquid chromatography/mass spectroscopy, and mass spectroscopy and antibody production by the hybridomas was assessed. *See* Example 6 and Figure 10.

In connection with functional analysis of antibodies in accordance with the present invention, such antibodies proved to be potent inhibitors of CTLA-4 30 and its binding to its ligands of the B7 family of molecules. For example, antibodies in accordance with the present invention were demonstrated to block CTLA-4 binding to either B7-1 or B7-2. *See* Example 7. Indeed, many of the

antibodies in accordance with the invention possess nanomolar and subnanomolar IC_{50} s with respect to inhibiting CTLA-4 binding to B7-1 and B7-2. Further, antibodies of the invention possess excellent selectivity for CTLA-4 as compared to CD28, CD44, B7-2, or hIgG1. *See* Example 8. Selectivity is a ratio that reflects the degree of preferential binding of a molecule with a first agent as compared to the molecules binding with a second, and optionally other molecules. Herein, selectivity refers to the degree of preferential binding of an antibody of the invention to CTLA-4 as compared to the antibody's binding to other molecules such as CD28, CD44, B7-2, or hIgG1. Selectivity values of antibodies of the invention greater than 500:1 are common. Antibodies of the invention have also been demonstrated to induce or enhance expression of certain cytokines (such as IL-2 and IFN- γ) by cultured T cells in a T cell blast model. *See* Examples 9 and 10 and Figures 12-17. Further, it is expected that antibodies of the invention will inhibit the growth of tumors in appropriate *in vivo* tumor models. The design of which models are discussed in Example 11 and 12.

The results demonstrated in accordance with the present invention indicate that antibodies of the present invention possess certain qualities that may make the present antibodies more efficacious than current therapeutic antibodies against CTLA-4.

In particular, the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention possess highly desirable properties. Their structural characteristics, functions, or activities provide criteria that facilitate the design or selection of additional antibodies or other molecules as discussed above. Such criteria include one or more of the following:

Ability to compete for binding to CTLA-4 with one or more of the antibodies of the invention;

Similar binding specificity to CTLA-4 as one or more of the antibodies of the invention;

A binding affinity for CTLA-4 of about 10^{-9} M or greater and preferably of about 10^{-10} M or greater;

Does not cross react with lower mammalian CTLA-4, including, preferably, mouse, rat, or rabbit and preferably mouse or rat CTLA-4;

Cross reacts with primate CTLA-4, including, preferably, cynomolgous and rhesus CTLA-4;

5 A selectivity for CTLA-4 over CD28, B7-2, CD44, or hIgG1 of at least about 100:1 or greater and preferably of about 300, 400, or 500:1 or greater;

An IC_{50} in blocking CTLA-4 binding to B7-2 of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.38 nM or lower;

10 An IC_{50} in blocking CTLA-4 binding to B7-1 of about of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.50 nM or lower;

An enhancement of cytokine production in one or more *in vitro* assays, for example:

15 An enhancement of IL-2 production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, 1500, 2000, 3000, or 3846 pg/ml or greater;

An enhancement of IFN- γ production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, or 1233 pg/ml or greater; or

20 An enhancement of IL-2 production in a hPBMC or whole blood superantigen assay of about 500 pg/ml or greater and preferably 750, 1000, 1200, or 1511 pg/ml or greater. Expressed another way, it is desirable that IL-2 production is enhanced by about 30, 35, 40, 45, 50 percent or more relative to control in the assay.

25 It is expected that antibodies (or molecules designed or synthesized therefrom) having one or more of these properties will possess similar efficacy to the antibodies described in the present invention.

30 The desirable functional properties discussed above can often result from binding to and inhibition of CTLA4 by a molecule (i.e., antibody, antibody fragment, peptide, or small molecule) in a similar manner as an antibody of the invention (i.e., binding to the same or similar epitope of the CTLA4 molecule).

The molecule may either be administered directly (i.e., direct administration to a patient of such molecules). Or, alternatively, the molecule may be “administered” indirectly (i.e., a peptide or the like that produces an immune response in a patient (similar to a vaccine) wherein the immune response includes the generation of antibodies that bind to the same or similar epitope or an antibody or fragment that is produced *in situ* after administration of genetic materials that encode such antibodies or fragments thereof which bind to the same or similar epitope). Thus, it will be appreciated that the epitope on CTLA4 to which antibodies of the invention bind to can be useful in connection with the preparation and/or design of therapeutics in accordance with the invention. In drug design, negative information is often useful as well (i.e., the fact that an antibody which binds to CTLA4 does not appear to bind to an epitope that acts as an inhibitor of CTLA4 is useful). Thus, the epitope to which antibodies of the invention bind that do not lead to the desired functionality can also be very useful. Accordingly, also contemplated in accordance with the present invention are molecules (and particularly antibodies) that bind to the same or similar epitopes as antibodies of the invention.

In addition to the fact that antibodies of the invention and the epitopes to which they bind are contemplated in accordance with the invention, we have conducted some preliminary epitope mapping studies of certain antibodies in accordance with the invention and particularly the 4.1.1 and the 11.2.1 antibodies of the invention.

As a first step, we conducted BIAcore competition studies to generate a rough map of binding as between certain antibodies of the invention in connection with their ability to compete for binding to CTLA4. To this end, CTLA4 was bound to a BIAcore chip and a first antibody, under saturating conditions, was bound thereto and competition of subsequent secondary antibodies binding to CTLA4 was measured. This technique enabled generation of a rough map in to which families of antibodies can be classified.

Through this process, we determined that the certain antibodies in accordance with the invention could be categorized as falling into the following epitopic categories:

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Category	Antibodies	Competition for CTLA4 Binding
A	BO1M*	Freely cross-compete with one another; cross-compete with category B; some cross-competition with category D
	BO2M**	
B	4.1.1	Freely cross-compete with one another; cross-compete with category A, C and D.
	4.13.1	
C	6.1.1	Freely cross-compete with one another; cross-compete with category B and category D
	3.1.1	
	4.8.1	
	11.2.1	
	11.6.1	
	11.7.1	
D	4.14.3	Cross-compete with category C and B; some cross-competition with category A
E	4.9.1	BNI3 blocks 4.9.1 binding to CTLA4 but not the reverse
	BNI3***	

(*) (**) Available from Biostride.

(***) Available from Pharmingen.

10 As a next step, we endeavored to determine if the antibodies of the invention recognized a linear epitope on CTLA4 under reducing and non-reducing conditions on Western blots. We observed that none of the 4.1.1, 3.1.1, 11.7.1, 11.6.1, or 11.2.1 antibodies of the invention appeared to recognize a reduced form of CTLA4 on Western blot. Accordingly, it appeared likely that
15 the epitope to which each of these antibodies bound was not a linear epitope but more likely was a conformational epitope the structure of which may have been abrogated under reducing conditions.

Therefore, we sought to determine whether we could learn about residues within the CTLA4 molecule that are important for binding of
20 antibodies of the invention. One manner that we utilized was to conduct kinetic assessments of off-rates as between human CTLA4 and two highly conserved primate CTLA4 molecules (cynomolgous and marmoset CTLA4). BIAcore

studies demonstrated that the 4.1.1 antibody of the invention bound to human, cynomologous, and marmoset CTLA4 at the same rate. However, with respect to off-rates (affinity), the 4.1.1 antibody had the highest affinity (slowest off-rate) for human, a faster off-rate with cynomologous, and a much faster off-rate for marmoset. The 11.2.1 antibody of the invention, on the other hand, binds to human, cynomologous, and marmoset CTLA4 at the about the same rate and has about the same relative off-rate for each of the three. This information further indicates that the 4.1.1 and 11.2.1 antibodies of the invention bind to different epitopes on CTLA4.

10 To further study the epitope to which the category B and C antibodies of the invention bind, we conducted certain site directed mutagenesis studies. Marmoset CTLA4 possesses two important changes at residues 105 and 106 relative to human CTLA4. Such differences are a leucine to methionine change at residue 105 and a glycine to serine change at residue 106. Accordingly, we
15 mutated cDNA encoding human CTLA4 to encode a mutated CTLA4 having the L105M and G106S changes. The homologue replacement mutant CTLA4 did not effect binding of a B7.2-IgG1 fusion protein. Further, binding with the 11.2.1 antibody of the invention was not effected. However, such molecule was significantly inhibited in its ability to bind with the 4.1.1 antibody of the
20 invention (similar to marmoset). Next, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a S106G change. Such change resulted in restoration of stable binding between the 4.1.1 antibody and the marmoset CTLA4 mutant. In addition, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a M105L
25 change. Such change partially restored binding between the 4.1.1 antibody and the mutant CTLA4.

Each of the category B through D antibodies of the invention appear to possess similar functional properties and appear to have the potential to act as strong anti-CTLA4 therapeutic agents. Further, each of the molecules certain
30 cross-competition in their binding for CTLA4. However, as will be observed from the above discussion, each of the molecules in the different categories appear to bind to separate conformational epitopes on CTLA4.

From the foregoing, it will be appreciated that the epitope information discussed above indicates that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention will likely have certain therapeutic potential in accordance with the present invention. Further, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) will likely have certain additional therapeutic potential in accordance with the present invention. Additionally, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) and that (i) are not reduced in their binding to marmoset CTLA4 (similar to the 11.2.1 antibody) or (ii) are reduced in their binding to marmoset CTLA4 (similar to the 4.1.1 antibody) will likely have certain additional therapeutic potential in accordance with the present invention. Antibodies (or other molecules, as discussed above) that compete with categories A and E may also have certain therapeutic potential.

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

EXAMPLE 1

Generation of Anti-CTLA-4-Antibody Producing Hybridomas

Antibodies of the invention were prepared, selected, and assayed in accordance with the present Example.

Antigen Preparation: Three distinct immunogens were prepared for immunization of the XenoMouseTM mice: (i) a CTLA-4-IgG fusion protein, (ii) a CTLA-4 peptide, and (iii) 300.19 murine lymphoma cells transfected with

a mutant of CTLA-4 (Y201V) that is constitutively expressed on the cell surface.

(i) CTLA-4-IgG1 Fusion Protein:

5

Expression Vector Construction:

The cDNA encoding the mature extracellular domain of CTLA-4 was PCR amplified from human thymus cDNA library (Clontech) using primers
 10 designed to published sequence (*Eur. J Immunol* 18:1901-1905 (1988)). The fragment was directionally subcloned into pSR5, a Sindbis virus expression plasmid (InVitrogen), between the human oncostatin M signal peptide and human IgG gamma 1 (IgG1) CH1/CH2/CH3 domains. The fusion protein does not contain a hinge domain but contains cysteine 120 in the extracellular
 15 domain of CTLA-4 to form a covalent dimer. The resulting vector was called CTLA-4-IgG1/pSR5. The complete CTLA-4-IgG1 cDNA in the vector was sequence confirmed in both strands. The amino acid sequence the CTLA4-Ig protein is shown below. The mature extracellular domain for CD44 was PCR amplified from human lymphocyte library (Clontech) and subcloned into
 20 pSinRep5 to generate a control protein with the identical IgG1 tail.

OM-CTLA4-IgG1 Fusion Protein:

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIASFVC
 25 EYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICT
GTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGINGTQIY
VIDPEPCPDSLEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPTPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 30 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Underlined: signal peptide

Bold: CTLA4 extracellular domain

5 The cDNAs for mature extracellular domain of CD28 were PCR amplified from human lymphocyte library (Clontech) and then subcloned into pCDM8 (*J. Immunol.* 151: 5261-71 (1993)) to produce a human IgG1 fusion protein containing both thrombin cleavage and hinge regions. Marmoset, Cynomologous, and Rhesus CTLA4 were cloned from mRNA isolated from
10 PHA stimulated PBMCs using standard techniques of degenerate PCR. Sequencing demonstrated that rhesus and cynomologous amino acid sequence were identical with three differences from mature human CTLA4 extracellular domain (S13N, I17T and L105M). Marmoset demonstrated ten amino acid differences from the mature human CTLA4 extracellular domain (V21A, V33I,
15 A41T, A51G, 54I, S71F, Q75K, T88M, L105M and G106S). Site directed mutagenesis was used to make single point mutations of all amino acids different in marmoset CTLA4 to map amino acids important for interaction of the antibodies with human CTLA4-IgG. Mutations of human and marmoset CTLA-IgG for epitope mapping were generated by matchmaker site-directed
20 mutagenesis (Promega). The IgG fusion proteins were produced by transient transfection of Cos7 cells and purified using standard Protein A techniques. Mutant CTLA4-IgG proteins were evaluated for binding to antibodies by immunoblotting and using BIAcore analyses.

25 Recombinant Protein Expression/Purification:

Recombinant sindbis virus was generated by electroporating (Gibco) Baby Hamster Kidney cells with SP6 *in vitro* transcribed CTLA-4-IgG1/pSR5 mRNA and DH-26S helper mRNA as described by InVitrogen. Forty eight
30 hours later recombinant virus was harvested and titered for optimal protein expression in Chinese hamster ovary cells (CHO-K1). CHO-K1 cells were cultured in suspension in DMEM/F12 (Gibco) containing 10% heat-inactivated

fetal bovine serum (Gibco), non-essential amino acids (Gibco), 4mM glutamine (Gibco), penicillin/streptomycin (Gibco), 10mM Hepes pH 7.5 (Gibco). To produce CTLA-4-IgG, the CHO-K1 cells were resuspended at 1×10^7 cells/ml in DMEM/F12 and incubated with sindbis virus for one hour at room temperature.

5 Cells were then diluted to 1×10^6 /ml in DMEM/F12 containing 1% fetal bovine serum depleted of bovine IgG using protein A sepharose (Pharmacia), non-essential amino acids, 4mM glutamine, 12.5mM Hepes pH 7.5, and penicillin/streptomycin. Forty eight hours post-infection cells were pelleted and conditioned media was harvested and supplemented with complete protease

10 inhibitor tablets (Boehringer Mannheim), pH adjusted to 7.5, and filtered 0.2μ (Nalgene). FPLC (Pharmacia) was used to affinity purify the fusion protein using a 5ml protein A HiTrap column (Pharmacia) at a 10ml/min flow rate. The column was washed with 30 bed volumes of PBS and eluted with 0.1M glycine/HCl pH 2.8 at 1ml/min. Fractions (1ml) were immediately

15 neutralized to pH 7.5 with Tris pH 9. The fractions containing CTLA-4-IgG1 were identified by SDS-PAGE and then concentrated using centrplus 50 (Amicon) before applying to sepharose 200 column (Pharmacia) at 1ml/min using PBS as the solvent. Fractions containing CTLA-4-IgG1 were pooled, sterile filtered 0.2μ (Millipore), aliquoted and frozen at -80°C . CD44-IgG1 was

20 expressed and purified using the same methods. CD28-IgG was purified from conditioned media from transiently transfected Cos7 cells.

Characterization CTLA-4-IgG1:

25 The purified CTLA-4-IgG1 migrated as a single band on SDS-PAGE using colloidal coomassie staining (Novex). Under non-reducing conditions CTLA-4-IgG1 was a dimer (100kDa), that reduced to a 50kDa monomer when treated with 50mM DTT. Amino acid sequencing of the purified CTLA-4-IgG1 in solution confirmed the N-terminus of CTLA-4 (MHVAQPAVVLAS), and

30 that the oncostatin-M signal peptide was cleaved from the mature fusion protein. The CTLA-4-IgG1 bound to immobilized B7.1-IgG in a concentration dependent manner and the binding was blocked by a hamster-anti-human anti-

CTLA-4 antibody (BNI3: PharMingen). The sterile CTLA-4-IgG was endotoxin free and quantitated by OD280 using 1.4 as the extinction coefficient. The yield of purified CTLA-4-IgG ranged between 0.5-3mgs/liter of CHO-K1 cells.

5

(ii) CTLA-4 Peptide:

The following CTLA-4 peptide was prepared as described below:

10 NH₂:MHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVT
EVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICK
VELMYPPPYLIGINGTQIYVIDPEPC-CONH₂

Abbreviations/Materials:

15

NMP, N-Methylpyrrolidinone; TFE, 2,2,2-Trifluoroethanol; DCM, Dichloromethane; Fmoc, Fluorenyl Methoxycarbonyl. All reagents were supplied by Perkin Elmer, with the following exceptions: TFE, Aldrich Chemical, Fmoc-PAL-PEG resin, Perseptive Biosystems. Fmoc-Arg(PMC)-
20 OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-His(Boc)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH and Fmoc-Tyr(tBu)-OH were used for those amino acids requiring side chain protecting groups

25

Peptide Synthesis:

Peptide synthesis was performed on a Perkin-Elmer 431A, retrofitted with feedback monitoring via UV absorbance at 301nm (Perkin-Elmer Model 759A detector). The peptide sequence was assembled on a Fmoc-PAL-PEG
30 resin using conditional double coupling cycles. Forced double couplings were performed at cycles 10,11,18,19,20 and 28 through 33. The resin was washed with a 50% mixture of DCM and TFE at the completion of each acylation cycle,

followed by capping of unreacted amino groups with acetic anhydride in NMP. Resin was removed from the reactor after completing cycle 49 and the remainder continued to completion. Peptide cleavage from the resin was performed using Reagent K (King et al. *International Journal of Protein and*
5 *Peptide Research* 36:255-266 (1990)) for 6 hours on 415mg of resin affording 186mg crude CTLA-4 peptide.

Peptide Characterization:

10 25mg aliquots of the crude CTLA-4 peptide were dissolved in 5ml 6M Guanidine HCl/100mM K₂PO₃ at pH6.4 and eluted over a Pharmacia Hi Load Superdex 75 16/60 column (16mm x 600mm, 120ml bed volume) with 2M Guanidine.HCl / 100mM K₂PO₃ at pH6.4 at 2 ml / min for 180 minutes collecting 5 ml fractions. The fractions were analyzed by loading 1.7µl of
15 fractions onto a NuPAGE Laemeli gel running with MES running buffer and visualizing via Daichii silver stain protocol. Those fractions exhibiting a molecular weight of 12 KDa, as judged versus molecular weight standards, were pooled together and stored at 4°C. The combined fractions were analyzed by UV and gel electrophoresis. Amino acid sequencing was performed by
20 absorbing a 100 microliter sample in a ProSorb cartridge (absorbed onto a PVDF membrane) and washing to remove the buffer salts. Sequencing was performed on an Applied Biosystems 420. The expected N-terminal sequence (M H V A Q P A V V L A) was observed. Immunoblotting demonstrated that the peptide was recognized by the BNI3 anti-human CTLA-4 (PharMingen).
25 To desalt, an aliquot containing 648µg of material was placed in 3500 Da MWCO dialysis tubing and dialyzed against 0.1% TFA / H₂O at 4°C for 9 days with stirring. The entire contents of the dialysis bag was lyophilized to a powder.

(iii) 300.19 cells transfected with CTLA-4 (Y201V)

The full length CTLA-4 cDNA was PCR amplified from human thymus cDNA library (Stratagene) and subcloned into pIRESneo (Clontech). A mutation of CTLA-4 that results in constitutive cell surface expression was introduced using MatchMaker Mutagenesis System (Promega). Mutation of tyrosine, Y201 to valine inhibits binding of the adaptin protein AP50 that is responsible for the rapid internalization of CTLA-4 (Chuang et al. *J. Immunol.* 159:144-151 (1997)). Mycoplasma-free 300.19 murine lymphoma cells were cultured in RPMI-1640 containing 10% fetal calf serum, non-essential amino acids, penicillin/streptomycin, 2mM glutamine, 12.5mM Hepes pH 7.5, and 25uM beta-mercaptoethanol. Cells were electroporated (3×10^6 /0.4ml serum free RPMI) in a 1ml chamber with 20ug CTLA-4-Y201V/pIRESneo using 200V/1180uF (Gibco CellPorator). Cells were rested for 10 minutes and then 8mls of prewarmed complete RPMI media. At 48 hours cells were diluted to 0.5×10^6 /ml in complete RPMI media containing 1mg/ml G418 (Gibco). Resistant cells were expanded and shown to express CTLA-4 on the cell surface using the BNI3 antibody conjugated with phycoerythrin (PharMingen). High level expressing cells were isolated by sterile sorting.

20

Immunization and hybridoma generation: XenoMouse mice (8 to 10 weeks old) were immunized (i) subcutaneously at the base of tails with 1×10^7 300.19 cells that were transfected to express CTLA-4 as described above, resuspended in phosphate buffered saline (PBS) with complete Freund's adjuvant, or (ii) subcutaneously at the base of tail with (a) 10 μ g the CTLA-4 fusion protein or (b) 10 μ g CTLA-4 peptide, emulsified with complete Freund's adjuvant. In each case, the dose was repeated three or four times in incomplete Freund's adjuvant. Four days before fusion, the mice received a final injection of the immunogen or cells in PBS. Spleen and/or lymph node lymphocytes from immunized mice were fused with the [murine non-secretory myeloma P3 cell line] and were subjected to HAT selection as previously described (Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and

30

procedures." *Methods Enzymol.* 73:3-46 (1981)). A large panel of hybridomas all secreting CTLA-4 specific human IgG₂κ or IgG₄κ (as detected below) antibodies were recovered.

5 **ELISA assay:** ELISA for determination of antigen-specific antibodies in mouse serum and in hybridoma supernatants was carried out as described (Coligan et al., Unit 2.1, "Enzyme-linked immunosorbent assays," in *Current protocols in immunology* (1994)) using CTLA-4-Ig fusion protein to capture the antibodies. For animals that are immunized with the CTLA-4-Ig fusion protein,
10 we additionally screen for non-specific reactivity against the human Ig portion of the fusion protein. This is accomplished using ELISA plates coated with human IgG1 as a negative control for specificity.

In a preferred ELISA assay, the following techniques are used:

ELISA plates are coated with 100 µl/well of the antigen in plate coating
15 buffer (0.1 M Carbonate Buffer, pH 9.6 and NaHCO₃ (MW 84) 8.4g/L). Plates are then incubated at 4°C overnight. After incubation, coating buffer is removed and the plate is blocked with 200 µl/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 hour. Alternatively, the plates are stored in refrigerator with
20 blocking buffer and plate sealers. Blocking buffer is removed and 50 µl/well of hybridoma supernatant, serum or other hybridoma supernatant (positive control) and HAT media or blocking buffer (negative control) is added. The plates are incubated at room temperature for 2 hours. After incubation, the plate is washed with washing buffer (1x PBS). The detecting antibody (i.e., mouse anti-
25 human IgG2-HRP (SB, #9070-05) for IgG2 antibodies or mouse anti-human IgG4-HRP (SB #9200-05) for IgG4 antibodies) is added at 100µl/well (mouse anti-human IgG2-HRP @ 1:2000 or mouse anti-human IgG4-HRP @ 1:1000 (each diluted in blocking buffer)). The plates are incubated at room temperature for 1 hour and then washed with washing buffer. Thereafter, 100 µl/well of
30 freshly prepared developing solution (10 ml Substrate buffer, 5 mg OPD (o-phenylenediamine, Sigma Cat No. P-7288), and 10 µl 30% H₂O₂ (Sigma)) is added to the wells. The plates are allowed to develop 10-20 minutes, until

negative control wells barely start to show color. Thereafter, 100 μ l/well of stop solution (2 M H_2SO_4) is added and the plates are read on an ELISA plate reader at wavelength 490 nm.

5 **Determination of affinity constants of fully human Mabs by BIAcore:**

Affinity measurement of purified human monoclonal antibodies, Fab fragments, or hybridoma supernatants by plasmon resonance was carried out using the BIAcore 2000 instrument, using general procedures outlined by the manufacturers.

10 Kinetic analysis of the antibodies was carried out using antigens immobilized onto the sensor surface at a low density. Three surfaces of the BIAcore sensorchip were immobilized with the CTLA-4-Ig fusion protein at a density ranging from approximately 390-900 using CTLA-4-Ig fusion protein at 20 or 50 μ g/ml in 10 mM sodium acetate at pH 5.0 using the amine coupling kit
15 supplied by the manufacturer (BIAcore, Inc.). The fourth surface of the BIAcore sensorchip was immobilized with IgG1 (900 RU) and was used as a negative control surface for non-specific binding. Kinetic analysis was performed at a flow rate of 25 or 50 microliters per minute and dissociation (k_d or k_{off}) and association (k_a or k_{on}) rates were determined using the software
20 provided by the manufacturer (BIA evaluation 3.0) that allows for global fitting calculations.

EXAMPLE 2

Affinity Measurement of Anti-CTLA-4-Antibodies

25

In the following Table, affinity measurements for certain of the antibodies selected in this manner are provided:

TABLE I

	Solid Phase (by BIAcore)				
Hybridoma	On-rates K_a ($M^{-1}S^{-1} \times 10^6$)	Off-rates K_d ($S^{-1} \times 10^{-4}$)	Association Constant $KA (1/M) =$ $k_a/k_d \times 10^{10}$	Dissociation Constant $KD(M) =$ $K_d/k_a \times 10^{-10}$	Surface Density [RU]
Moab01	0.68	1.01	0.67	1.48	878.7
	0.70	4.66	0.15	6.68	504.5
	0.77	6.49	0.19	8.41	457.2
	0.60	3.08	0.20	5.11	397.8
4.1.1	1.85	0.72	2.58	0.39	878.7
	1.88	1.21	1.55	0.64	504.5
	1.73	1.54	1.13	0.88	457.2
	1.86	1.47	1.26	0.79	397.8
4.8.1	0.32	0.07	4.46	0.22	878.7
	0.31	0.23	1.33	0.75	504.5
	0.28	0.06	4.82	0.21	397.8
4.14.3	2.81	3.04	0.92	1.08	878.7
	2.88	3.97	0.73	1.38	504.5
	2.84	6.66	0.43	2.35	457.2
	3.17	5.03	0.63	1.58	397.8
6.1.1	0.43	0.35	1.21	0.83	878.7
	0.46	0.90	0.51	1.98	504.5
	0.31	0.51	0.61	1.63	457.2
	0.45	0.79	0.57	1.76	397.8
3.1.1	1.04	0.96	1.07	0.93	878.7
	0.95	1.72	0.55	1.82	504.5
	0.73	1.65	0.44	2.27	457.2
	0.91	2.07	0.44	2.28	397.8
4.9.1	1.55	13.80	0.11	8.94	878.7
	1.43	19.00	0.08	13.20	504.5
	1.35	20.50	0.07	15.20	397.8
4.10.2	1.00	2.53	0.39	2.54	878.7
	0.94	4.30	0.22	4.55	504.5
	0.70	5.05	0.14	7.21	457.2
	1.00	5.24	0.19	5.25	397.8
2.1.3	1.24	9.59	0.13	7.72	878.7
	1.17	13.10	0.09	11.20	504.5
	1.11	13.00	0.09	11.70	397.8
4.13.1	1.22	5.83	0.21	4.78	878.7
	1.29	6.65	0.19	5.17	504.5
	1.23	7.25	0.17	5.88	397.8

As will be observed, antibodies prepared in accordance with the invention possess high affinities and binding constants.

5

EXAMPLE 3

Structures of Anti-CTLA-4-Antibodies Prepared in Accordance with the Invention

In the following discussion, structural information related to antibodies
10 prepared in accordance with the invention is provided.

In order to analyze structures of antibodies produced in accordance with the invention, we cloned genes encoding the heavy and light chain fragments out of the particular hybridoma. Gene cloning and sequencing was accomplished as follows:

15 Poly(A)⁺ mRNA was isolated from approximately 2 X 10⁵ hybridoma cells derived from immunized XenoMouse mice using a Fast-Track kit (Invitrogen). The generation of random primed cDNA was followed by PCR. Human V_H or human V_κ family specific variable region primers (Marks et al., "Oligonucleotide primers for polymerase chain reaction amplification of human
20 immunoglobulin variable genes and design of family-specific oligonucleotide probes." *Eur. J. Immunol.* 21:985-991 (1991)) or a universal human V_H primer, MG-30 (CAGGTGCAGCTGGAGCAGTCIGG) was used in conjunction with primers specific for the human Cγ2 constant region (MG-40d; 5'-GCTGAGGGAGTAGAGTCCTGAGGA-3') or Cκ constant region (hκP2;
25 as previously described in Green et al., 1994). Sequences of human Mabs-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly(A⁺) RNA using the primers described above. PCR products were also cloned into pCRII using a TA cloning kit (Invitrogen) and both strands were sequenced using
30 Prism dye-terminator sequencing kits and an ABI 377 sequencing machine. All sequences were analyzed by alignments to the "V BASE sequence directory"

(Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK) using MacVector and Geneworks software programs.

Further, each of the antibodies 4.1.1, 4.8.1, 11.2.1, and 6.1.1 were subjected to full length DNA sequences. For such sequencing, Poly(A)⁺ mRNA was isolated from approximately 4 X 10⁶ hybridoma cells using mRNA Direct kit (Dynal). The mRNA was reverse transcribed using oligo-dT(18) and the Advantage RT/PCR kit (Clontech). The Variable region database (V Base) was used to design amplification primers beginning at the ATG start site of the heavy chain DP50 gene (5'-
 10 TATCTAAGCTTCTAGACTCGACCGCCACCATGGAGTTTGGGCTGAGC
 TG-3') and to the stop codon of the IgG2 constant region (5'-
 TTCTCTGATCAGAATTCCTATCATTTACCCGGAGACAGGGAGAGCT-
 3'). An optimal Kozak sequence (ACCGCCACC) was added 5' to the ATG start site. The same method was used to design a primer to the ATG start site of
 15 the kappa chain A27 gene (5'-
 TCTTCAAGCTTGCCCGGGCCCGCCACCATGGAAACCCAGCGCAG
 -3') and the stop codon of the kappa constant region (5'-
 TTCTTTGATCAGAATTCTCACTAACACTCTCCCCTGTTGAAGC-3').
 The 012 cDNA was cloned by using a primer to the ATG start site (5'-
 20 TCTTCAAGCTTGCCCGGGCCCGCCACCATGGACATGAGGGTCCCCGC
 T-3) and the kappa constant region stop codon primer above. The heavy chain cDNAs were also cloned as genomic constructs by site directed mutagenesis to add an NheI site at the end of the variable J domain and subcloning an NheI-
 fragment containing the genomic IgG2 CH1/Hinge/CH2/CH3 regions. The
 25 point mutation to generate NheI site does not alter the amino acid sequence from germline. The primer pairs were used to amplify the cDNAs using Advantage High Fidelity PCR Kit (Clontech). Sequence of the PCR was obtained by direct sequencing using dye-terminator sequencing kits and an ABI sequencing machine. The PCR product was cloned into pEE glutamine
 30 synthetase mammalian expression vectors (Lonza) and three clones were sequenced to confirm somatic mutations. For each clone, the sequence was verified on both strands in at least three reactions. An aglycosylated 4.1.1

antibody was generated by site directed mutagenesis of N294Q in the CH2 domain. Recombinant antibodies were produced by transient transfection of Cos7 cells in IgG depleted FCS and purified using standard Protein A sepharose techniques. Stable transfectants were generated by electroporation of murine
5 NSO cells and selection in glutamine free media. Recombinant 4.1.1 with or without glycosylation exhibited identical specificity and affinity for CTLA4 in the *in vitro* ELISA and BIAcore assays.

Gene Utilization Analysis

10

The following Table sets forth the gene utilization evidenced by selected hybridoma clones of antibodies in accordance with the invention:

TABLE II
Heavy and Light Chain Gene Utilization

Clone	Heavy Chain				Kappa Light Chain	
	VH	D	JH		VK	JK
4.1.1	DP-50	DIR4 or DIR3	JH4		A27	JK1
4.8.1	DP-50	7-27	JH4		A27	JK4
4.14.3	DP-50	7-27	JH4		A27	JK3
6.1.1	DP-50	DIR5 or DIR5rc	JH4		A27	JK3
3.1.1	DP-50	3-3	JH6		012	JK3
4.10.2	DP-50	7-27	JH4		A27	JK3
2.1.3	DP-65	1-26	JH6		A10/A26	JK4
4.13.1	DP-50	7-27	JH4		A27	JK3
11.2.1	DP-50	D1-26	JH6		012	JK3
11.6.1	DP-50	D2-2 or D4	JH6		012	JK3
11.7.1	DP-50	D3-22 or D21-9	JH4		012	JK3
12.3.1.1	DP-50	D3-3 or DXP4	JH6		A17	JK1
12.9.1.1	DP-50	D6-19	JH4		A3/A19	JK4
4.9.1	DP-47	5-24 and/or 6-19	JH4		L5	JK1

5

As will be observed, antibodies in accordance with the present invention were generated with a strong bias towards the utilization of the DP-50 heavy chain variable region. The DP-50 gene is also referred to as a V_H 3-33 family gene. Only one antibody that was selected on the basis of CTLA-4 binding and preliminary *in vitro* functional assays showed a heavy chain gene utilization other than DP-50. That clone, 2.1.3, utilizes a DP-65 heavy chain variable region and is an IgG4 isotype. The DP-65 gene is also referred to as a V_H 4-31 family gene. On the other hand, the clone, 4.9.1, which possesses a DP-47 heavy chain variable region binds to CTLA-4 but does not inhibit binding to B7-1 or B7-2. In XenoMouse mice, there are more than 30 distinct functional

15

heavy chain variable genes with which to generate antibodies. Bias, therefore, is indicative of a preferred binding motif of the antibody-antigen interaction with respect to the combined properties of binding to the antigen and functional activity.

5

Mutation Analysis

As will be appreciated, gene utilization analysis provides only a limited overview of antibody structure. As the B-cells in XenoMouse animals stochastically generate V-D-J heavy or V-J kappa light chain transcripts, there are a number of secondary processes that occur, including, without limitation, somatic hypermutation, n-additions, and CDR3 extensions. *See, for example,* Mendez et al. *Nature Genetics* 15:146-156 (1997) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996. Accordingly, to further examine antibody structure predicted amino acid sequences of the antibodies were generated from the cDNAs obtained from the clones. In addition, N-terminal amino acid sequences were obtained through protein sequencing.

Figure 1 provides nucleotide and predicted amino acid sequences of the heavy and kappa light chains of the clones 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M). In Figures 1A, 1B, and 1D, extended sequences of the antibodies 4.1.1, 4.8.1, and 6.1.1 were obtained by full length cloning of the cDNAs as described above. In such Figures, the signal peptide sequence (or the bases encoding the same) are indicated in bold and sequences utilized for the 5' PCR reaction are underlined.

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The

Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "Os".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17 amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the

positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains:

4.1.1:

full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c));

full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e));

4.1.1 light chain (cDNA 22(f) and amino acid 22(g));

4.8.1:

full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i));

4.8.1 light chain (cDNA 22(j) and amino acid 22(k));

6.1.1:

full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m));

6.1.1 light chain (cDNA 22(n) and amino acid 22(o));

11.2.1:

full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)); and

11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

Signal peptide sequences are shown in bold and large text. The open reading frames in the full length 4.1.1 genomic DNA sequence (Fig. 22(b)) are underlined. And, the mutations introduced to make the aglycosylated 4.1.1 heavy chain and the resulting change (N294Q) are shown in double underline and bold text (cDNA (Fig. 22(b)) and amino acid (Fig. 22(c))).

EXAMPLE 4

Analysis of Heavy and Light Chain Amino Acid Substitutions

10 In Figure 2, which provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence, an interesting pattern emerges. In addition to the fact of the bias for heavy chain DP-50 in the majority of the clones, there is relatively limited hypermutation in the antibodies relative to the germline DP-50 gene. For example, clones 3.1.1 and 11.2.1 have no mutations. Moreover, the mutations in the other clones are generally conservative changes, involving substitutions of amino acids with similar properties to the amino acids in the germline. Mutations within many of the CDR1 and CRD2 sequences are particularly conservative in nature. Three of the heavy chains represented in Figure 2, 4.10.2, 4.13.1, and 4.14.3, are clearly derived from a single recombination event (i.e., derive from an identical germinal center) and are nearly identical in sequence. If these three are considered as a single sequence, then, among the 10 different antibodies containing the DP50 heavy chain, in CDR1 and CDR2 there are 3 positions in which a nonpolar residue is replaced by another nonpolar residue, 12 in which a polar uncharged residue is replaced by another polar uncharged residue, and 1 in which a polar charged residue is replaced by another polar charged residue. Further, there are two positions in which two residues which are very similar structurally, glycine and alanine, are substituted for one another. The only mutations not strictly conservative involve 3 substitutions of a polar charged residue for a polar uncharged residue and one substitution of a nonpolar residue for a polar residue.

The light chains of these antibodies are derived from 5 different Vk genes. The A27 gene is the most heavily represented and is the source of 6 different light chains. Comparison of these 6 sequences reveals two noteworthy features. First, in three of them, 4.8.1, 4.14.3, and 6.1.1, contain deletions of one or two residues in CDR1, a rare event. Second, there is a strong prejudice against the germline serine at position six in CDR3 in that the serine has been replaced in every sequence. This suggests that a serine at this position is incompatible with CTLA4 binding.

It will be appreciated that many of the above-identified amino acid substitutions exist in close proximity to or within a CDR. Such substitutions would appear to bear some effect upon the binding of the antibody to the CTLA-4 molecule. Further, such substitutions could have significant effect upon the affinity of the antibodies.

EXAMPLE 5

N-Terminal Amino Acid Sequence Analysis of Antibodies in Accordance with the Invention

In order to further verify the composition and structure of the antibodies in accordance with the invention identified above, we sequenced certain of the antibodies using a Perkin-Elmer sequencer. Both heavy and kappa light chains of the antibodies were isolated and purified through use of preparative gel electrophoresis and electroblotting techniques and thereafter directly sequenced as described in Example 6. A majority of the heavy the heavy chain sequences were blocked on their amino terminus. Therefore, such antibodies were first treated with pyroglutamate aminopeptidase and thereafter sequenced.

The results from this experiment are shown in Figure 9. Figure 9 also provides the molecular weight of the heavy and light chains as determined by mass spectroscopy (MALDI).

EXAMPLE 6**Additional Characterization of Antibodies in Accordance with the
Invention**

5 Figure 10 provides certain additional characterizing information about certain of the antibodies in accordance with the invention. In the Figure, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. The following data is provided: Concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, FACS, mass spectroscopy (MALDI),
10 and light chain N-terminal sequences.

 Generally, the data was generated as follows:

Materials and Methods

15 Protein concentration was determined at 280 nm from a UV scan (200-350 nm), where 1.58 absorbance units at 280 nm equaled 1mg/ml.

SDS-PAGE was performed using the Novex NuPAGE electrophoresis system with a 10% NuPAGE gel and MES running buffer. Samples were prepared by diluting 3:1 with 4x NuPAGE sample buffer (+/-)
20 beta-mercaptoethanol, heated and ~ 5 ug of protein was loaded onto the gel. The gel was then stained with Brilliant Blue R staining solution (Sigma cat.# B-6529) and molecular size estimates were made by comparing stained bands to "Perfect Protein Markers" (Novagen cat# 69149-3).

25

 For N-terminal sequencing, samples were run as above on NuPAGE gels, transferred to Pro Blot immobilization membrane (Applied Biosystems) then stained with Coomassie Blue R-250. The stained protein bands were excised and subjected to sequence analysis by automated Edman degradation on
30 an Applied Biosystems 494 Procise HT Sequencer.

Isoelectric focusing (IEF) was performed using Pharmacia IEF 3-9 pHast gels (cat# 17-0543-01) . Samples were diluted in 10% glycerol to ~0.8 mg/ml and 1ul was loaded onto gel and then silver stained. The pI estimates were made by comparing stained bands to broad range (pH3-10) IEF standards
5 (Pharmacia cat # 17-0471-01)

Size exclusion chromatography (SEC) was carried in phosphate buffered saline (PBS) on the Pharmacia SMART system using the Superdex 75 PC 3.2/30 column. Molecular size estimates were made by comparing peak
10 retention time to the retention times of gel

For FACS studies, human peripheral T cells were prepared and stimulated for 48 hours. T cells were washed once, resuspended in FACS buffer at 1×10^6 cells/100 ul and stained for CD3 surface expression with 10 ul
15 of anti-CD3-FITC (Immunotech, Marseille, France) for 30 minutes at room temperature. Cells were washed twice, then fixed, permeabilized (Fix and Perm, Caltag), and stained for intracellular CTLA-4 expression with 10 ul anti-CD152-PE (Pharmingen). Flow cytometry was performed using a Becton Dickinson FACSort. Quadrants were set by analysis of relevant isotype control
20 antibodies (Caltag).

As was discussed above, anti-CTLA-4 antibodies have been demonstrated to possess certain powerful immune modulation activities. The
25 following experiments were carried out in order to determine if antibodies in accordance with the present invention possessed such activities. In general, the experiments were designed to assess ability of the antibodies to inhibit the interaction between CTLA-4 and B7 molecules, be selective as between CTLA-4 and B7 molecules and CD28, and promote T cell cytokine production,
30 including, but not limited to IL-2 and/or IFN- γ expression. Further, examination of cross-reactivity of antibodies of the invention with certain

human tissues and CTLA-4 molecules in other species (e.g., mouse and primate) was undertaken.

EXAMPLE 7

5 Competition ELISA: Inhibition of CTLA-4/B7-1 or B7-2 Interaction by Antibodies in Accordance with the Invention

An *in vitro* assay was conducted to determine if antibodies in accordance with the present invention were capable of inhibiting the binding of
10 CTLA-4 with either B7-1 or B7-2. As will be appreciated, antibodies of the invention that are capable of inhibiting the binding of CTLA-4 with B7 molecules would be expected to be candidates for immune regulation through the CTLA-4 pathway. In the assay, the following materials and methods were utilized:

15

Materials and Methods

3 nM B7.1-Ig(G1) or B7.2-Ig(G1) (Repligen, Inc. Needham, MA) in Dulbecco's PBS was coated on 96-well MaxiSorp plates (Nunc, Denmark,
20 #439454) and incubated at 4°C overnight. On day 2, B7-Ig was removed and plates were blocked with 1% BSA plus 0.05% Tween-20 in D-PBS for two hours. Plates were washed 3X with wash buffer (0.05% Tween-20 in D-PBS). Antibody at appropriate test concentrations and CTLA-4-Ig(G4) (0.3 nM final conc.) (Repligen, Inc. Needham, MA) were pre-mixed for 15 minutes and then
25 added to the B7-Ig coated plate (60 ul total volume) and incubated at RT for 1.5 hours. Plates were washed 3X and 50 µl of a 1 to 1000 dilution of HRP-labeled mouse anti-human IgG4 antibody (Zymed, San Francisco, CA, #05-3820) was added and incubated at RT for 1 hour. Plates were washed 3X and 50 µl TMB
30 Microwell peroxidase substrate (Kirkegaard & Perry, Gaithersburg, MD, #50-76-04) was added and incubated at RT for 20 minutes, and then 50 µl 1N H₂SO₄ was added to the plate. Plates were read at 450 nm using a Molecular Devices plate reader (Sunnyvale, CA). All samples were tested in duplicate. Maximal

signal was defined as CTLA-4-Ig binding in the absence of test antibody. Non-specific binding was defined as absorbance in the absence of CTLA-4-Ig and test antibody.

- 5 The results from the assay are provided in Table IIIA and IIIB. In Table IIIA, results are shown for a variety of antibodies in accordance with the invention. In Table IIIB, results are shown comparing the 4.1.1 antibody of the invention with the 11.2.1 antibody of the invention from a separate experiment.

10

TABLE IIIA

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT3.1.1	IgG2	0.45 ± 0.07 (n=3)	0.63 ± 0.10 (n=2)
CT4.1.1	IgG2	0.38 ± 0.06 (n=5)	0.50 ± 0.05 (n=2)
CT4.8.1	IgG2	0.57 ± 0.03 (n=3)	0.17 ± 0.28 (n=2)
CT4.9.1	IgG2	Non-competitive (n=3)	non-competitive (n=2)
CT4.10.2	IgG2	1.50 ± 0.37 (n=3)	3.39 ± 0.31 (n=2)
CT4.13.1	IgG2	0.49 ± 0.05 (n=3)	0.98 ± 0.11 (n=2)
CT4.14.3	IgG2	0.69 ± 0.11 (n=3)	1.04 ± 0.15 (n=2)
CT6.1.1	IgG2	0.39 ± 0.06 (n=3)	0.67 ± 0.07 (n=2)

15

TABLE IIIB

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT4.1.1	IgG2	0.55 ± 0.08 (n=4)	0.87 ± 0.14 (n=2)
CT11.2.1	IgG2	0.56 ± 0.05 (n=4)	0.81 ± 0.24 (n=2)

EXAMPLE 8**Selectivity Ratios of Antibodies of the Invention with Respect to CTLA-4
Versus Either CD28 or B7-2**

5 Another *in vitro* assay was conducted to determine the selectivity of antibodies of the invention with respect to CTLA-4 versus either CD28 or B7-2. The following materials and methods were utilized in connection with the experiments:

10 **CTLA-4 Selectivity ELISA: Materials and Methods**

A 96-well FluroNUNC plate (Nunc Cat No.475515) was platecoated with four antigens: CTLA-4/Ig, CD44/Ig, CD28/Ig, and B7.2/Ig (antigens generated in-house). The antigens were platecoated overnight at +4°C at 1ug/ml
15 100ul/well in 0.1M sodium bicarbonate buffer, pH 9.6. The plate was then washed with PBST (PBS + 0.1% Tween-20) three times using a NUNC plate washer. The plate was blocked with PBST+0.5%BSA at 150 ul/well. The plate was incubated at RT for 1 hour then washed with PBST three times. Next the anti-CTLA-4 antibodies of the invention were diluted in block at 1 µg/ml and
20 were added to the plate. The plate was incubated at RT for 1 hour then washed with PBST three times. The wells that contained the antibodies of the invention were then treated with 100 µl/well anti-human IgG2-HRP (Southern Biotech Cat No.9070-05) at a 1:4000 dilution in block. Also, one row was treated with anti-human IgG (Jackson Cat No. 209-035-088) to normalize for platecoating.
25 This antibody was diluted to 1:5000 in block and added at 100 ul/well. Also, one row was treated with anti-human CTLA-4-HRP (Pharmingen Cat No. 345815/Custom HRP conjugated) as a positive control. This antibody was used at 0.05 ug/ml diluted in block. The plate was incubated at RT for 1 hour then washed with PBST three times. LBA chemiluminescent substrate (Pierce) was
30 added at 100 µl/well and the plate was incubated on a plateshaker for 5 min. The plate was then read using a lumi-imager for a 2 min. exposure.

IGEN CTLA-4-Ig Selectivity Binding Assay: Materials and Methods

M-450 Dynabeads (Dynal A.S, Oslo, Norway #140.02) were washed 3X with Na phosphate buffer, pH 7.4 and resuspended in Na phosphate buffer. 1.0 μ g CTLA-4-Ig(G1), 1.0 μ g CD28-Ig(G1) or 1.0 to 3.0 μ g B7.2-Ig(G1) (Repligen, Inc. Needham, MA) were added to 100 μ l of beads and incubated overnight on a rotator at 4°C. On day 2 the beads were washed 3X in 1% BSA plus 0.05% Tween-20 in Dulbecco's PBS and blocked for 30 minutes. Beads were diluted 1 to 10 with blocking buffer and 25 μ l of the coated beads were added to 12x75 mm polypropylene tubes. All samples were tested in duplicate. 50 μ l test antibody (1 μ g/ml final concentration) or blocking buffer was added to the tubes and incubated for 30 minutes on the Origen 1.5 Analyzer carousel (IGEN International, Inc., Gaithersburg, MD) at RT, vortexing at 100 rpm. 25 μ l of ruthenylated murine anti-human IgG1, IgG2 or IgG4 (Zymed, Inc. San Francisco, CA #05-3300, 05-3500 and 05-3800) (final concentration of 3 μ g/ml in 100 μ l total volume) was added to the tubes. Tubes were incubated for 30 minutes at RT on the carousel vortexing at 100 rpm. 200 μ l of Origen assay buffer (IGEN International, Inc., Gaithersburg, MD #402-050-03) per tube was added and briefly vortexed and then the tubes were counted in the Origen Analyzer and ECL (electrochemiluminescence) units were determined for each tube. Normalization factors were determined to correct for differences in binding of fusion proteins to Dynabeads, and ECL units were corrected for non-specific binding before calculating selectivity ratios.

The results from the assays are provided in Tables IVA and IVB.

TABLE IVA

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/CD44 ELISA	CTLA4/CD28 IGEN	CTLA4/B7.2 IGEN
3.1.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=1) 195:1 (n=1)
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 485:1 (n=1)	>500:1 (n=3)	>500:1 (n=1) 261:1 (n=1)	>500:1 (n=1) 107:1 (n=1)
4.8.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 190:1 (n=1)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)
4.9.1	IgG2	>500:1 (n=2) 244:1 (n=1)	>500:1 (n=2) 33:1 (n=1)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.10.2	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.13.1	IgG2	>500:1 (n=2) 46:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1) 329:1 (n=1)	>500:1 (n=2)
4.14.3	IgG2	>500:1 (n=2) 80:1 (n=1)	>500:1 (n=2) 10:1 (n=1)	>500:1 (n=2) 126:1 (n=1)	>413:1 (n=1)	>234:1 (n=1)
6.1.1	IgG2	>500:1 (n=2) 52:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)

5

TABLE IVB

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/hIgG ELISA
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=3)
11.2.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)

10

EXAMPLE 9**Human T-Cell Signal Model**

15

In order to further define the activity of antibodies in accordance with the invention to act as immune regulators, we developed certain T-cell assays in order to quantify the enhancement of T-cell IL-2 production upon blockade of

CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:

Materials and Methods

5

Freshly isolated human T cells were prepared by using Histopaque (Sigma, St. Louis, MO #A-70543) and T-kwik (Lympho-Kwik, One Lambda, Canoga Park, CA, #LK-50-T), and stimulated with PHA (1 μ g/ml) (Purified Phytohemagglutinin, Murex Diagnostics Ltd. Dartford, England, #HA 16) in
10 medium (RPMI 1640 containing L-glutamine, MEM non-essential amino acids, penicillin, streptomycin, 25 mM Hepes and 10% FBS) at a concentration of 1×10^6 cells/ml and incubated at 37°C for 2 days. The cells were washed and diluted in medium to 2×10^6 cells/ml. Raji cells (Burkitt lymphoma, Human ATCC No.: CCL 86 Class II American Type Culture Collection Rockville, MD)
15 were treated with mitomycin C (Sigma St. Louis, MO, # M-4287) (25 μ g/ml) for one hour at 37°C. The Raji cells were washed 4X in PBS and resuspended at 2×10^6 cells/ml. Human T cell blasts (5×10^5 /ml), Raji cells (5×10^5 /ml) and anti-CTLA-4 antibodies or an isotype-matched control antibody at various concentrations were added to 96-well microtiter plates and the plates were
20 incubated at 37°C for 72 hours. Total volume per well was 200 μ l. Seventy-two hours post stimulation, the plates were spun down and supernatant removed and frozen for later determination of IL-2 (Quantikine IL-2 ELISA kit, R&D Systems, Minneapolis, MN, #D2050) and IFN- γ (Quantikine IFN-g ELISA kit, R&D Systems). Cytokine enhancement was defined as the difference between
25 cytokine levels in cultures containing an anti-CTLA-4 blocking mAb versus an isotype-matched control antibody. For flow cytometry experiments, Raji cells were washed 1x with FACS buffer (PBS containing 2% heat inactivated FCS, 0.025% sodium azide). Cell pellets were resuspended in FACS buffer at 1×10^6 cells/100 μ l and incubated with 10 μ l of anti-CD80-PE (Becton Dickinson, San
30 Jose, CA) or anti-CD86-PE (Pharmingen, San Diego, CA) for 30 minutes at room temperature. Cells were washed twice and resuspended in 1 ml FACS buffer. Flow cytometry was performed using a Becton Dickinson FACSsort.

Histogram markers were set by analysis of relevant isotype control antibodies (Caltag, Burlingame, CA).

In general, we have developed an assay that can be used for rapid
5 determination of T-cell IL-2 upregulation. As will be appreciated, stimulation of T cells is B7 and CD28 dependent. Further, washed T blasts do not make detectable IL-2 and Raji cells do not make detectable IL-2 even when stimulated with LPS or PWM. However, in combination, the T blasts co-cultured with Raji cells can model B7, CTLA-4, and CD28 signaling events and
10 the effects of antibodies thereon can be assessed.

Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs using flow cytometry (FACs) as described in Example 6.

Figure 12 shows the concentration dependent enhancement of IL-2
15 production in the T cell blast/Raji assay induced by CTLA-4 blocking antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention).

Figure 13 shows the concentration dependent enhancement of IFN- γ production in the T cell blast/Raji assay induced by CTLA-4 blocking
20 antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) (same donor T cells).

Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay. It is interesting to consider that the mAb, CT4.9.1, binds to CTLA4 but
25 does not block B7 binding. Thus, simply binding to CTLA-4 is insufficient by itself to provide a functional antibody of the invention.

Figure 15 shows the mean enhancement of IFN- γ production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay.

30 Figure 19 shows a comparison between the 4.1.1 and 11.2.1 antibodies of the invention at a concentration of 30 μ g/ml in the 72 hour T cell blast/Raji

assay as described in this Example 9 and the Superantigen assay described in Example 10.

Figure 20 shows the concentration dependent enhancement of IL-2 production in the T cell blast/Raji assay induced by the 4.1.1 and 11.2.1 CTLA4 antibodies of the invention.

The following Table IVc provides information related to mean enhancement and range of enhancement of cytokine response in the Raji and SEA assays of the invention. Each of the experiments included in the results are based on antibody at a dose of 30 µg/ml and measured at 72 hours. Numbers of donors used in the experiments as well as responses are shown.

TABLE IVc

Assay	mAb	Cytokine	Mean Enhancement pg/ml	SEM	Range Enhancement pg/ml	n	Donor Response
T cell blast/Raji	4.1.1	IL-2	3329	408	0 to 8861	42	19 of 21
T cell blast/Raji	4.1.1	IFN-γ	3630	980	600 to 13939	17	13 of 13
T cell blast/Raji	11.2.1	IL-2	3509	488	369 to 6424	18	14 of 14
SEA (PBMC)	4.1.1	IL-2	2800	312	330 to 6699	42	17 of 17
SEA (PBMC)	11.2.1	IL-2	2438	366	147 to 8360	25	15 of 15
SEA (Whole Blood)	4.1.1	IL-2	6089	665	-168 to 18417	46	15 of 17
SEA (Whole Blood)	11.2.1	IL-2	6935	700	-111 to 11803	25	12 of 14

EXAMPLE 10

Human T-Cell Signal Model

We developed a second cellular assay in order to quantify the enhancement of T-cell IL-2 upregulation upon blockade of CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:

Materials and Methods

Human PBMC were prepared using Accuspin. Microtiter plates were precoated with an anti-CD3 antibody (IeD4, Becton Dickinson) (60 ng/ml) and incubated for 2 hours at 37°C. hPBMC were added to the wells at 200,000 cells per well. Staphylococcus enterotoxin A (SEA) (Sigma) was added to the wells at 100 ng/ml. Antibodies were added to the wells, usually at 30 µg/ml. Cells were then stimulated for 48, 72 or 96 hours. Plates were centrifuged at the desired time-point and supernatants were removed from the wells. Thereafter, supernatants were checked for IL-2 production using ELISA (R&D Systems).

Results from these experiments are shown in Figures 16, 17, and 21. In Figure 16, induction of IL-2 production in hPBMC from 5 donors was measured 72 hours after stimulation. In Figure 17, results are shown from measurement of whole blood, analyzing the difference in induction of IL-2 production in the blood of 3 donors as measured at 72 and 96 hours after stimulation.

In Figure 21, the enhancement of IL-2 production in whole blood of 2 donors as measured at 72 hours after stimulation.

EXAMPLE 11

Tumor Animal Model

We have established an animal tumor model for the *in vivo* analysis of anti-murine-CTLA-4 antibodies in inhibiting tumor growth. In the model, a murine fibrosarcoma tumor is grown and the animals are treated with anti-murine-CTLA-4 antibodies. The materials and methods for establishment of the model are provided below:

Materials and Methods

Female A/J mice (6-8 weeks old) were injected subcutaneously on the dorsal side of the neck with 0.2 ml of Sa1N tumor cells (1×10^6) (Baskar 1995).

Anti-murine CTLA-4 or an isotype matched control antibody (PharMingen, San Diego, CA, 200 ug/animal) were injected intraperitoneally on days 0, 4, 7 and 14 following the injection of tumor cells. Tumor measurements were taken during the course of the 3-4 week experiments using a Starrett SPC Plus
5 electronic caliper (Athol, MA) and tumor size was expressed as the surface area covered by tumor growth (mm²).

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model. As shown in Figure 18, animals treated with anti-CTLA-4 had a reduction in tumor growth as
10 compared to animals treated with an isotype control antibody. Accordingly, anti-murine CTLA4 mAbs are capable of inhibiting growth of a fibrosarcoma in a mouse tumor model.

It is expected that antibodies that are cross-reactive with murine CTLA-4 would perform similarly in the model. However, of the antibodies of the
15 invention that have been checked for cross-reactivity, none are cross-reactive with murine CTLA-4.

EXAMPLE 12

Tumor Animal Model

20

In order to further investigate the activity of antibodies in accordance with the invention, a xenograft SCID mouse model was designed to test the eradication of established tumors and their derived metastases. In the model, SCID mice are provided with grafted human T cells and are implanted with
25 patient-derived non-small cell lung cell (NSCL) or colorectal carcinoma (CC) cells. Implantation is made into the gonadal fat pads of SCID mice. The tumors are allowed to grow, and thereafter removed. The mice develop human-like tumor and liver metastases. Such a model is described in Bumpers et al *J. Surgical Res.* 61:282-288 (1996).

30

It is expected that antibodies of the invention will inhibit growth of tumors formed in such mice.

INCORPORATION BY REFERENCE

All references cited herein, including patents, patent applications,
5 papers, text books, and the like, and the references cited therein, to the extent
that they are not already, are hereby incorporated herein by reference in their
entirety. In addition, the following references are also incorporated by
reference herein in their entirety, including the references cited in such
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EQUIVALENTS

5 The foregoing description and Examples detail certain preferred
embodiments of the invention and describes the best mode contemplated by the
inventors. It will be appreciated, however, that no matter how detailed the
foregoing may appear in text, the invention may be practiced in many ways and
the invention should be construed in accordance with the appended claims and
10 any equivalents thereof.

CLAIMSWHAT WE CLAIM IS:

5 1. An antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V_H3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or
10 framework sequences shown in Figure 2.

 2. The antibody of Claim 1, wherein the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6,
15 SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70.

 3. The antibody of Claim 1, further comprising a light chain
20 variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,
25 and SEQ ID NO:71.

 4. The antibody of Claim 2, further comprising a light chain
variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15,
30 SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ

ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,
and SEQ ID NO:71.

5 5. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:1 and a light chain variable amino acid sequence
comprising SEQ ID NO:14.

6. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:2 and a light chain variable amino acid sequence
10 comprising SEQ ID NO:15.

7. An antibody comprising a heavy chain amino acid sequence
comprising SEQ ID NO:4 and a light chain variable amino acid sequence
comprising SEQ ID NO:17.

15

8. A isolated human monoclonal antibody that is capable of binding
to CTLA-4.

9. The antibody of Claim 8, wherein the antibody is capable of
20 competing for binding with CTLA-4 with an antibody selected from the group
consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,
11.7.1, 12.3.1.1, and 12.9.1.1.

10. The antibody of Claim 8, wherein the antibody possesses a
25 substantially similar binding specificity to CTLA-4 as an antibody selected from
the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

11. The antibody of Claim 8, wherein the antibody is selected from
30 the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

12. The antibody of Claim 8, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

13. The antibody of Claim 12, wherein the lower mammalian species
5 comprises mouse, rat, and rabbit.

14. The antibody of Claim 8, wherein the antibody is cross reactive with CTLA-4 from primates.

10 15. The antibody of Claim 14, wherein the primates comprise cynomolgous and rhesus monkeys.

16. The antibody of Claim 8, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than
15 about 100:1.

17. The antibody of Claim 16, wherein the selectivity is about 500:1 or greater.

20 18. The antibody of Claim 8, wherein a binding affinity of the antibody is about 10^{-9} M or greater.

19. The antibody of Claim 18, wherein a binding affinity of the antibody is about 10^{-10} M or greater.

25

20. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM.

21. The antibody of Claim 20, wherein the antibody inhibits binding
30 between CTLA-4 and B7-2 with an IC_{50} of lower than about 0.38 nM.

22. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater.

23. The antibody of Claim 22, wherein the antibody inhibits binding
5 between CTLA-4 and B7-1 with an IC_{50} of lower than about 0.50 nM.

24. The antibody of Claim 8, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

10 25. The antibody of Claim 24, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

26. The antibody of Claim 8, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater.

15

27. The antibody of Claim 26, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

28. The antibody of Claim 8, wherein the antibody induces IL-2
20 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater.

29. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml
25 or greater

30. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

30

31. The antibody of Claim 26, wherein the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.
- 5 32. A humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.
- 10 33. The antibody of Claim 32, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.
34. The antibody of Claim 33, wherein the lower mammalian species comprises mouse, rat, and rabbit.
- 15 35. The antibody of Claim 32, wherein the antibody is cross reactive with CTLA-4 from primates.
36. The antibody of Claim 35, wherein the primates comprise cynomolgous and rhesus monkeys.
- 20 37. The antibody of Claim 32, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1.
- 25 38. The antibody of Claim 37, wherein the selectivity is about 500:1 or greater.
39. The antibody of Claim 32, wherein a binding affinity of the antibody is about 10^{-9} M or greater.
- 30

40. The antibody of Claim 39, wherein a binding affinity of the antibody is about 10^{-10} M or greater.

41. The antibody of Claim 32, wherein the antibody inhibits binding
5 between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM.

42. The antibody of Claim 41, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 0.38 nM.

10 43. The antibody of Claim 32, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater.

44. The antibody of Claim 43, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 0.50 nM.

15

45. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

46. The antibody of Claim 45, wherein the antibody enhances IL-2
20 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

47. The antibody of Claim 32, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 500 pg/ml or greater.

25 48. The antibody of Claim 47, wherein the antibody enhances IFN- γ production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

49. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml
30 or greater.

50. The antibody of Claim 49, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml or greater

5 51. The antibody of Claim 32, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

10 52. The antibody of Claim 50, wherein the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

15 53. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2.

20 54. The antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

25 55. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties:

a binding affinity for CTLA-4 of about 10^{-9} or greater;

inhibits binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower;

30 inhibits binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and

enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

56. An antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties:

a binding affinity for CTLA-4 of about 10^{-9} or greater;
10 inhibits binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower;
inhibits binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and
enhances cytokine production in an assay of human T cells by
15 500 pg/ml or greater.

57. A cell culture system for assaying T cell stimulation, comprising a culture of human T cell blasts co-cultured with a Raji cell line.

20 58. The cell culture system of Claim 57, wherein the T cell blasts are washed prior to culture with the Raji cell line.

59. An assay for measuring T cell stimulation, comprising:
providing a culture of human T cell blasts and a Raji cell line;
25 contacting the culture with an agent; and
measuring cytokine production by the culture.

60. The assay of Claim 59, wherein the T cell blasts are washed prior to culture with the Raji cell line.

30

61. The assay of Claim 59, wherein the cytokine is IL-2.

62. The assay of Claim 59, wherein the cytokine is IFN- γ .
63. The assay of Claim 59, wherein cytokine production is measured in supernatant isolated from the culture.
- 5 64. The assay of Claim 59, wherein the agent is an antibody.
65. The assay of Claim 59, wherein the antibody binds to CTLA-4.
- 10 66. A functional assay for screening a moiety for T cell stimulatory function, comprising:
providing a culture of human T cell blasts and a Raji cell line;
contacting the culture with the moiety; and
assessing cytokine production by the culture.
- 15 67. The assay of Claim 66, wherein the T cell blasts are washed prior to culture with the Raji cell line.
68. The assay of Claim 66, wherein the cytokine is IL-2.
- 20 69. The assay of Claim 66, wherein the cytokine is IFN- γ .
70. The assay of Claim 66, wherein cytokine production is assessed in supernatant isolated from the culture.
- 25 71. The assay of Claim 66, wherein the moiety is an antibody.
72. The assay of Claim 66, wherein the antibody binds to CTLA-4.
- 30 73. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

74. The assay of Claim 73, wherein the T cell blasts are washed prior to culture with the Raji cell line.
- 5 75. The assay of Claim 73, wherein the cytokine is IL-2.
76. The assay of Claim 73, wherein the cytokine is IFN- γ .
77. The assay of Claim 73, wherein cytokine production is assessed
10 in supernatant isolated from the culture.
78. The assay of Claim 73, wherein the agent is an antibody.
79. The assay of Claim 73, wherein the antibody binds to CTLA-4.
15
80. A method for screening an agent for T cell stimulatory activity, comprising:
contacting the agent with a cell culture comprising human T cell
blasts and a Raji cell line; and
20 assessing cytokine production by the culture.
81. The method of Claim 80, wherein the T cell blasts are washed prior to culture with the Raji cell line.
- 25 82. The method of Claim 80, wherein the cytokine is IL-2.
83. The method of Claim 80, wherein the cytokine is IFN- γ .
84. The method of Claim 80, wherein cytokine production is
30 assessed in supernatant isolated from the culture.

85. The method of Claim 80, wherein the agent is an antibody that binds to CTLA-4.

86. An assay for measuring T cell stimulation, comprising:
5 providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;
contacting the culture with an agent; and
measuring cytokine production by the cell population.

10

87. The assay of Claim 86, wherein the cytokine is IL-2.

88. The assay of Claim 86, wherein cytokine production is measured in supernatant isolated from the cell population.

15

89. The assay of Claim 86, wherein the agent is an antibody.

90. The assay of Claim 86, wherein the antibody binds to CTLA-4.

20

91. A functional assay for screening a moiety for T cell stimulatory function, comprising:

providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A;
25 contacting the culture with the moiety; and
assessing cytokine production by the cell population.

92. The assay of Claim 91, wherein the cytokine is IL-2.

30 93. The assay of Claim 91, wherein cytokine production is assessed in supernatant isolated from the cell population.

94. The assay of Claim 91, wherein the moiety is an antibody .

95. The assay of Claim 91, wherein the antibody binds to CTLA-4.

5 96. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.

10 97. The assay of Claim 96, wherein the cytokine is IL-2.

98. The assay of Claim 96, wherein cytokine production is assessed in supernatant isolated from the cell population.

15 99. The assay of Claim 96, wherein the agent is an antibody.

100. The assay of Claim 96, wherein the antibody binds to CTLA-4.

20 101. A method for screening an agent for T cell stimulatory activity, comprising:

 contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and
 assessing cytokine production by the cell population.

25

102. The method of Claim 101, wherein the cytokine is IL-2.

103. The method of Claim 101, wherein cytokine production is assessed in supernatant isolated from the cell population.

30

104. The method of Claim 101, wherein the agent is an antibody that binds to CTLA-4.

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Figure 1A**4.1.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTTT TAAGAGGTGT 50
CCAGTGTCAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTGTAGCGT CTGGATTCAC CTTCAGTAGC 150
CATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGAAATAA ATACTATGCA GACTCCGTGA 250
AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTTTCTG 300
CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGGAGGTCAC TTCGGTCCTT TTGACTACTG GGGCCAGGGA ACCCTGGTCA 400
CCGTCTCCTC AGCCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCGCCC 450
TGCTCCAGGA GCACCTCCGA GAGCACAGCG GCCCTGGGCT GCCTGGTCAA 500
GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA GCGCTCTGA 550
CCAGCGGCGT GCACACCTTC CCAGCTGTCC TACAGTCCTC AGGACTCTAC 600
TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAACTTCG GCACCCAGAC 650
CTACACCTGC AACGTAGATC ACAAGCCCAG CAACACCAAG GTGGACAAGA 700
CAGTTGAGCG CAAATGTTGT GTCGAGTGCC CACCGTGCCC AGCACCACCT 750
GTGGCAGGAC CGTCAGTCTT CCTCTTCCCC CAAAACCCA AGGACACCCT 800
CATGATCTCC CGGACCCCTG AGGTCACGTG CGTGGTGGTG GACGTGAGCC 850
ACGAAGACCC CGAGGTCCAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG 900
CATAATGCCA AGACAAAGCC ACGGGAGGAG CAGTTCAACA GCACGTTCCG 950
TGTGGTCAGC GTCCTCACC GGTGACCA GGACTGGCTG AACGGCAAGG 1000
AGTACAAGTG CAAGGTCTCC AACAAAGGCC TCCAGCCCC CATCGAGAAA 1050
ACCATCTCCA AAACCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT 1100
GCCCCATCC CGGGAGGAGA TGACCAAGAA CCAGGTCAGC CTGACCTGCC 1150
TGGTCAAAGG CTTCTACCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT 1200
GGGCAGCCGG AGAACAATA CAAGACCACA CCTCCCATGC TGGACTCCGA 1250
CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC 1300
AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC 1350
CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA 1392

```

(SEQ ID NO:27)

4.1.1 Heavy Chain Protein

```

MEFGLSWVFL VALLRGVQCQ VQLVESGGGV VQPGRSLRLS CVASGFTFSS 50
HGMHWVRQAP GKLEWVAVI WYDGRNKYYA DSVKGRFTIS RDNSKNTLFL 100
QMNSLRAEDT AVYYCARGGH FGPFYWGQG TLVTVSSAST KGPSVFPLAP 150
CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 200
SLSSVTVPS SNFGTQTYTC NVDHKPSNTK VDKTVERKCC VECPPCPAPP 250
VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FNWYVDGVEV 300
HNAKTKPREE QFNSTFRVVS VLTIVHQDWL NGKEYKCKVS NKGLPAPIEK 350
TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN 400
GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 450
HYTQKSLSL PGK 463

```

(SEQ ID NO:1)

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Figure 1A (continued)**4.1.1 Kappa Chain DNA**

```

ATGGAAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGCA GGGCCAGTCA GAGTATTAGC 150
AGCAGCTTCT TAGCCTGGTA CCAGCAGAGA CCTGGCCAGG CTCCCAGGCT 200
CCTCATCTAT GGTGCATCCA GCAGGGCCAC TGGCATCCCA GACAGGTTCA 250
GTGGCAGTGG GTCTGGGACA GACTTCACTC TCACCATCAG CAGACTGGAG 300
CCTGAAGATT TTGCAGTGTA TTACTGTCAG CAGTATGGTA CCTCACCCTG 350
GACGTTCCGC CAAGGGACCA AGGTGGAAAT CAAACGAACT GTGGCTGCAC 400
CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAAC 450
GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAG AGGCCAAAGT 500
ACAGTGGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG 550
TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCTG 600
ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT 650
CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG 700
AGTGTTAG 708

```

(SEQ ID NO:40)

4.1.1 Kappa Chain Protein

```

METPAQLLFL LLLWLPD TTG EIVLTQSPGT LSLSPGERAT LSCRASQ SIS 50
SSFLAWYQQR PGQAPRL LIY GASSRATGIP DRFSGSGSGT DFTLTISRLE 100
PEDFAVYYCQ QYGTSPWTFG QGTKVEIKRT VAAPSVFIFP PSDEQLKSGT 150
ASVVCLLN NF YPREAKVQWK VDNLQSGNS QESVTEQDSK DSTYLSSTL 200
TLISKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEC 235

```

(SEQ ID NO:14)

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Figure 1B**4.8.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTTT TAAGAGGTGT 50
CCAGTGTCTAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTACAGCGT CTGGATTAC CTTAGTAAC 150
TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGTAATAA AACTATGGA GACTCCGTGA 250
AGGGCCGATT CACCATCTCC AGTGACAATT CCAAGAACAC GCTGTATCTG 300
CAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGGAGAGAGA CTGGGGTCCT ACTTTGACTA CTGGGGCCAG GGAACCCTGG 400
TCACCGTCTC CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCTGGCG 450
CCCTGCTCCA GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT 500
CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC 550
TGACCAGCGG CGTGACACAC TTCCAGCTG TCCTACAGTC CTCAGGACTC 600
TACTCCCTCA GCAGCGTGGT GACCGTGCCC TCCAGCAACT TCGGCACCCA 650
GACCTACACC TGCAACGTAG ATCACAAGCC CAGCAACACC AAGGTGGACA 700
AGACAGTTGA GCGCAAATGT TGTGTCGAGT GCCCACCCTG CCCAGACCA 750
CCTGTGGCAG GACCGTCAGT CTTCTCTTC CCCCCAAAAC CCAAGGACAC 800
CCTCATGATC TCCCGGACCC CTGAGGTCAC GTGCGTGGTG GTGGACGTGA 850
GCCACGAAGA CCCCAGAGTC CAGTTCAACT GGTACGTGGA CGGCGTGGAG 900
GTGCATAATG CCAAGACAAA GCCACGGGAG GAGCAGTTCA ACAGCACGTT 950
CCGTGTGGTC AGCGTCCTCA CCGTTGTGCA CCAGGACTGG CTGAACGGCA 1000
AGGAGTACAA GTGCAAGGTC TCCAACAAAG GCCTCCCAGC CCCATCGAG 1050
AAAACCATCT CCAAAACCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC 1100
CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC AGCCTGACCT 1150
GCCTGGTCAA AGGCTTCTAC CCCAGCGACA TCGCCGTGGA GTGGGAGAGC 1200
AATGGGCAGC CGGAGAACAA CTACAAGACC ACACCTCCCA TGCTGGACTC 1250
CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT 1300
GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC 1350
AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGA 1395

```

(SEQ ID NO:28)

4.8.1 Heavy Chain Protein

```

MEFGLSWVFL VALLRGVQCQ VQLVESGGGV VQPGSLRLS CTASGFTFSN 50
YGMHWVRQAP GKLEWVAVI WYDGSNKHYG DSVKGRFTIS SDNSKNTLYL 100
QMNSLRAEDT AVYYCARGER LGSYFDYWQ GTLVTVSSAS TKGPSVFPLA 150
PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 200
YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVKRC CVECPCPAP 250
PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE 300
VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE 350
KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES 400
NGQPENNYKT TTPMLDSGGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH 500
NHYTQKSLSL SPGK 514

```

(SEQ ID NO:2)

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Figure 1B (continued)4.8.1 Kappa Chain DNA

```

ATGGAAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGCA GGACCAGTGT TAGCAGCAGT 150
TACTTAGCCT GGTACCAGCA GAAACCTGGC CAGGCTCCCA GGCTCCTCAT 200
CTATGGTGCA TCCAGCAGGG CCACTGGCAT CCCAGACAGG TTCAGTGGCA 250
GTGGGTCTGG GACAGACTTC ACTCTACCA TCAGCAGACT GGAGCCTGAA 300
GATTTTGCAG TCTATTACTG TCAGCAGTAT GGCATCTCAC CCTTCACTTT 350
CGGCGGAGGG ACCAAGGTGG AGATCAAGCG AACTGTGGCT GCACCATCTG 400
TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT 450
GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG 500
GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTACAG 550
AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG 600
AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA 650
TCAGGGCCTG AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT 700
AG 702

```

(SEQ ID NO:41)

4.8.1 Kappa Chain Protein

```

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRTSVSSS 50
YLAWYQQKPG QAPRLLIYGA SSRATGIPDR FSGSGSGTDF TLTIISRLPE 100
DFAVYYCQY GISPFTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS 150
VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSTLTL 200
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC 233

```

(SEQ ID NO:15)

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Figure 1C**4.14.3 Heavy Chain DNA**

```

CCTGGGAGGT CCCTGAGACT CTCCTGTGCA GCGTCTGGAT TCACCTTCAG 50
TAGTCATGGC ATCCACTGGG TCCGCCAGGC TCCAGGCAAG GGGCTGGAGT 100
GGGTGGCAGT TATATGGTAT GATGGAAGAA ATAAAGACTA TGCAGACTCC 150
GTGAAGGGCC GATTCACCAT CTCCAGAGAC AATTCCAAGA AGACGCTGTA 200
TTTGCAAATG AACAGCCTGA GAGCCGAGGA CACGGCTGTG TATTACTGTG 250
CGAGAGTGGC CCCACTGGGG CCACTTGACT ACTGGGGCCA GGGAAACCCTG 300
GTCACCGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 350
GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCGGCCCTG GGCTGCCTGG 400
TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCT 450
CTGACCAGCG GCGTGCACAC CTTCCCAGCT GTCCTACAG 489

```

(SEQ ID NO:29)

4.14.3 Heavy Chain Protein

```

PGRSLRLSCA ASGFTFSSHG IHWVRQAPGK GLEWVAVIWY DGRNKDYADS 50
VKGRFTISRD NSKKTLYLQM NSLRAEDTAV YYCARVAPLG PLDYWGQGT 100
VTVSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA 150
LTSGVHTFPA VLQ 163

```

(SEQ ID NO:3)

4.14.3 Kappa Chain DNA

```

GGCACCCCTGT CTTTGTCTCC AGGGGAAAGA GCCACCCTCT CCTGCAGGGC 50
CAGTCAGAGT GTCAGCAGCT ACTTAGCCTG GTACCAGCAG AAACCTGGCC 100
AGGCTCCCAG ACTCCTCATC TATGGTGCAT CCAGCAGGGC CACTGGCATC 150
CCAGACAGGT TCAGTGGCAG TGGGTCTGGG ACAGACTTCA CTCTCACCAT 200
CAGCAGACTG GAGCCTGAGG ATTTTGCAGT GTATTACTGT CAGCAGTATG 250
GTAGGTCACC ATTCAC TTTC GGCCCTGGGA CCAAAGTGGA TATCAAGCGA 300
ACTGTGGCTG CACCATCTGT CTTTCATCTC CCGCCATCTG ATGAGCAGTT 350
GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA 400
GAGAGGCCAA AGTACAG 417

```

(SEQ ID NO:42)

4.14.3 Kappa Chain Protein

```

GTLSLSPGER ATLSCRASQS VSSYLAWYQQ KPGQAPRLLI YGASSRATGI 50
PDRFSGSGSG TDFLTISRL EPEDFAVYYC QQYGRSPFTF GPGTKVDIKR 100
TVAAPS VFIF PPSDEQLKSG TASVVC LLNN FYPREKVQ 139

```

(SEQ ID NO:16)

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Figure 1D**6.1.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTTT TAAGAGGTGT 50
CCAGTGTCAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCGAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTACAGCGT CTGGATTAC CTTCAGTAGT 150
TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGCAATAA ACACTATGCA GACTCCGCGA 250
AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG 300
CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGCCGGACTG CTGGGTACTT TTGACTACTG GGGCCAGGGA ACCCTGGTCA 400
CCGTCTCCTC AGCCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCGCCC 450
TGCTCCAGGA GCACCTCCGA GAGCACAGCG GCCCTGGGCT GCCTGGTCAA 500
GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA GGCCTCTGTA 550
CCAGCGGCGT GCACACCTTC CCAGCTGTCC TACAGTCCTC AGGACTCTAC 600
TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAACTTCG GCACCCAGAC 650
CTACACCTGC AACGTAGATC ACAAGCCCAG CAACACCAAG GTGGACAAGA 700
CAGTTGAGCG CAAATGTTGT GTCGAGTGCC CACCGTGCCC AGCACCACCT 750
GTGGCAGGAC CGTCAGTCTT CCTCTTCCCC CCAAACCCA AGGACACCCT 800
CATGATCTCC CGGACCCCTG AGGTCACGTG CGTGGTGGTG GACGTGAGCC 850
ACGAAGACCC CGAGGTCCAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG 900
CATAATGCCA AGACAAAGCC ACGGGAGGAG CAGTTCAACA GCACGTTCCG 950
TGTGGTCAGC GTCCTCACCG TTGTGCACCA GGACTGGCTG AACGGCAAGG 1000
AGTACAAGTG CAAGGTCTCC AACAAAGGCC TCCCAGCCCC CATCGAGAAA 1050
ACCATCTCCA AAACCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT 1100
GCCCCATCC CGGGAGGAGA TGACCAAGAA CCAGGTCAGC CTGACCTGCC 1150
TGGTCAAAGG CTTCTACCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT 1200
GGGCAGCCGG AGAACAATA CAAGACCACA CCTCCCATGC TGGACTCCGA 1250
CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC 1300
AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC 1350
CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA 1392

```

(SEQ ID NO:30)

6.1.1 Heavy Chain Protein

```

MEFGLSWVFL VALLRGVQCQ VOLVESGGGV VEPGRSLRLS CTASGFTFSS 50
YGMHWVRQAP GKGLEWVAVI WYDGSNKHYA DSAKGRFTIS RDNSKNTLYL 100
QMNSLRAEDT AVYYCARAGL LGYFDYWQQG TLVTVSSAST KGPSVFPLAP 150
CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 200
SLSSVVTGPS SNFGTQTYTC NVDHKPSNTK VDKTVERKCC VECPPCPAPP 250
VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FNWYVDGVEV 300
HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS NKGLPAPIEK 350
TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN 400
GQPENNYKTT PPM L DSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 450
HYTQKSLSL PGK 463

```

(SEQ ID NO:4)

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Figure 1D (continued)6.1.1 Kappa Chain DNA

```

ATGGAAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGTA GGGCCAGTCA AAGTGTTAGC 150
AGCTACTTAG CCTGGTACCA ACAGAAACCT GGCCAGGCTC CCAGGCCCCCT 200
CATCTATGGT GTATCCAGCA GGGCCACTGG CATCCCAGAC AGGTTTCAGTG 250
GCAGTGGGTC TGGGACAGAC TTCACTCTCA CCATCAGCAG ACTGGAGCCT 300
GAAGATTTTG CAGTGTATTA CTGTCAGCAG TATGGTATCT CACCATTAC 350
TTTCGGCCCT GGGACCAAAG TGGATATCAA ACGAACTGTG GCTGCACCAT 400
CTGTCTTCAT CTTCCCGCCA TCTGATGAGC AGTTGAAATC TGGAAGTACC 450
TCTGTTGTGT GCCTGCTGAA TAACCTTCTAT CCCAGAGAGG CCAAAGTACA 500
GTGGAAGGTG GATAACGCCC TCCAATCGGG TAACTCCCAG GAGAGTGTCA 550
CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCTGACG 600
CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC 650
CCATCAGGGC CTGAGCTCGC CCGTCACAAA GAGCTTCAAC AGGGGAGAGT 700
GTTAG 705

```

(SEQ ID NO:43)

6.1.1 Kappa Chain Protein

```

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRASQSVS 50
SYLAWYQQKP GQAPRPLIYG VSSRATGIPD RFSGSGSGTD FTLTISRLEP 100
EDFAVYQCQ YGISPFTEFGP GTKVDIKRTV AAPSVFIFPP SDEQLKSGTA 150
SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT 200
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK 234

```

(SEQ ID NO:17)

8/48

Figure 1E**3.1.1.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTACACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTACAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAGG GCCCGTATAA TAACCCCTTG TATGGACGTC 300
TGGGGCCAAG GGACCACGGT CACCGTCTCC TCAGCCTCCA CCAAGGGCCC 350
ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG 400
CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG 450
TCGTGGAAC T CAGGCGCTCT GACCAGCGGC GTGCACACCT TCCCAGCTGT 500
CCTACAG 507

```

(SEQ ID NO:31)

3.1.1.1 Heavy Chain Protein

```

GVVQPGRSLR LS CAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARG ARIITPCMDV 100
WGQGTITVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV 150
SWNSGALTSG VHTFPAVLQ 169

```

(SEQ ID NO:5)

3.1.1.1 Kappa Chain DNA

```

CAGTCTCCAT CCTCCCTGTC TGCATCTGTA GGAGACAGAG TCACCATCAC 50
TTGCCGGGCA AGTCAGAGCA TTAACACCTA TTAAATTTGG TATCAGCAGA 100
AACCAGGGAA AGCCCCTAAC TTCCTGATCT CTGCTACATC CATTTTGCAA 150
AGTGGGGTCC CATCAAGGTT CCGTGGCAGT GGCTCTGGGA CAAATTTTAC 200
TCTCACCATC AACAGTCTTC ATCCTGAAGA TTTTGCAACT TACTACTGTC 250
AACAGAGTTA CAGTACCCCA TTCACCTTCG GCCCTGGGAC CAAAGTGGAT 300
ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA 450
TCGGGTAA 458

```

(SEQ ID NO:44)

3.1.1.1 Kappa Chain Protein

```

QSPSSLSASV GDRVITTCRA SQSINTYLIW YQQKPGKAPN FLISATSIQ 50
SGVPSRFRGS GSGTNFTLTI NSLHPEDFAT YYCQQSYSTP FTFGPGTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ 150
SG 152

```

(SEQ ID NO:18)

9/48

Figure 1F**4.10.2 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG TAGCGTCTGG 50
ATTCATCTTC AGTAGTCATG GCATCCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG AAATAAAGAC 150
TATGCAGACT CCGTGAAGGG CCGATTACAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATTTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCGAGAGTG GCCCCACTGG GGCCACTTGA CTACTGGGGC 300
CAGGGAACCC TGGTCACCGT CTCCTCAGCC TCCACCAAGG GCCCATCGGT 350
CTTCCCCCTG GCGCCCTGCT CCAGGAGCAC CTCCGAGAGC ACAGCGGCCC 400
TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG 450
AACTCAGGCG CTCTGACCAG CGGCGTGCAC ACCTTCCCAG CTGTCCTACA 500
G 501

```

(SEQ ID NO:32)

4.10.2 Heavy Chain Protein

```

GVVQPGRSLR LSCVASGFIF SSHGIHWVRQ APGKGLEWVA VIWYDGRNKD 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARV APLGPLDYWG 100
QGTLVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD YFPEPVTVSW 150
NSGALTSGVH TFPVQLQ 167

```

(SEQ ID NO:6)

4.10.2 Kappa Chain DNA

```

TCTCCAGGCA CCCTGTCTTT GTCTCCAGGG GAAAGAGCCA CCCTCTCCTG 50
CAGGGCCAGT CAGAGTATTA GCAGCAATTT CTTAGCCTGG TACCAGCAGA 100
AACCTGGCCA GGCTCCCAGG CTCCTCATCT ATCGTCCATC CAGCAGGGCC 150
ACTGGCATCC CAGACAGTTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC 200
TCTCACCATC AGCAGACTGG AGCCTGAGGA TTTTGCATTA TATTACTGTC 250
AGCAGTATGG TACGTCACCA TTCACTTTCG GCCCTGGGAC CAAAGTGGAT 300
ATCAAGCGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCCAG AGAGGCCAAA GTACAG 426

```

(SEQ ID NO:45)

4.10.2 Kappa Chain Protein

```

SPGTLSLSPG ERATLSCRAS QSISSNFLAW YQKPGQAPR LLIYRPSSRA 50
TGIPDSFSGS GSGTDFTLTI SRLEPEDFAL YYCQQYGTSP FTFGPGTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQ 142

```

(SEQ ID NO:19)

10/48

Figure 1G**2.1.3 Heavy Chain DNA**

```

TCGGGCCCCAG GACTGGTGAA GCCTTCACAG ATCCTGTCCC TCACCTGCAC 50
TGTCTCTGGT GGCTCCATCA GCAGTGGTGG TCACTACTGG AGCTGGATCC 100
GCCAGCACCC AGGGAAGGGC CTGGAGTGGA TTGGGTACAT CTATTACATT 150
GGGAACACCT ACTACAACCC GTCCCTCAAG AGTCGAGTTA CCATATCAGT 200
AGACACGTCT AAGAACCAGT TCTCCCTGAA GCTGAGCTCT GTGACTGCCG 250
CGGACACGGC CGTGTATTAT TGTGCGAGAG ATAGTGGGGA CTACTACGGT 300
ATAGACGTCT GGGGCCAAGG GACCACGGTC ACCGTCTCCT CAGCTTCCAC 350
CAAGGGCCCA TCCGTCTTCC CCCTGGCGCC CTGCTCCAGG AGCACCTCCG 400
AGAGCACAGC CGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG 450
GTGACGGTGT CGTGGAAGTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT 500
CCCGGCTGTC CTACAA 516

```

(SEQ ID NO:33)

2.1.3 Heavy Chain Protein

```

SGPGLVKPSQ ILSLTCTVSG GSISSGGHYW SWIRQHPGKG LEWIGYIYYI 50
GNTYYNPSLK SRVTISVDTS KNQFSLKLSS VTAADTAVYY CARDSGDYYG 100
IDVWGQGTTV TVSSASTKGP SVFPLAPCSR STSESTAALG CLVKDYFPEP 150
VTVSWNSGAL TSGVHTFPAV LQ 172

```

(SEQ ID NO:7)

2.1.3 Kappa Chain DNA

```

TCTCCAGACT TTCAGTCTGT GACTCCAAAG GAGAAAGTCA CCATCACCTG 50
CCGGGCCAGT CAGAGCATTG GTAGTAGCTT ACATTGGTAT CAGCAGAAAC 100
CAGATCAGTC TCCAAAGCTC CTCATCAAGT ATGCTTCCCA GTCCTTCTCT 150
GGGGTCCCCCT CGAGGTTTCA TGGCAGTGGA TCTGGGACAG ATTTACCCTT 200
CACCATCAAT AGCCTGGAAG CTGAAGATGC TGCAACGTAT TACTGTCATC 250
AGAGTAGTAG TTTACCGCTC ACTTTCGGCG GAGGGACCAA GGTGGAGATC 300
AAACGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC CATCTGATGA 350
GCAGTTGAAA TCTGGAAGTG CCTCTGTTGT GTGCCTGCTG AATAACTTCT 400
ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG 450
GGTAACTCCC AGGAG 465

```

(SEQ ID NO:46)

2.1.3 Kappa Chain Protein

```

SPDFQSVTPK EKVTITCRAS QSIGSSLHWY QOKPDQSPKL LIKYASQSFS 50
GVPSRFSGSG SGTDFTLTIN SLEAEDAATY YCHQSSSLPL TFGGGTKVEI 100
KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREKAV QWKVDNALQS 150
GNSQE 155

```

(SEQ ID NO:20)

11/48

Figure 1H**4.13.1 Heavy Chain DNA**

```

CCTGGGAGGT CCCTGAGACT CTCCTGTGCA GCGTCTGGAT TCACCTTCAG 50
TAGTCATGGC ATCCACTGGG TCCGCCAGGC TCCAGGCAAG GGGCTGGAGT 100
GGGTGGCAGT TATATGGTAT GATGGAAGAA ATAAAGACTA TGCAGACTCC 150
GTGAAGGGCC GATTCACCAT CTCCAGAGAC AATTCCAAGA ACACGCTGTA 200
TTTGCAAATG AACAGCCTGA GAGCCGAGGA CACGGCTGTG TATTACTGTG 250
CGAGAGTGGC CCCACTGGGG CCACTTGACT ACTGGGGCCA GGGAAACCCTG 300
GTCACCGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 350
GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCGGCCCTG GGCTGCCTGG 400
TCAAGGACTA CTCCCCGAA CCGGTGACGG TGTCTGGAA CTCAGGCGCT 450
CTGACCAGC 459

```

(SEQ ID NO:34)

4.13.1 Heavy Chain Protein

```

PGRSLRLSCA ASGFTFSSHG IHWVRQAPGK GLEWVAVIWY DGRNKDYADS 50
VKGRFTISR D NSKNTLYLQM NSLRAEDTAV YYCARVAPLG PLDYWGQGT 100
VTVSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA 150
LTS 153

```

(SEQ ID NO:8)

4.13.1 Kappa Chain DNA

```

CAGTCTCCAG GCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC 50
CTGCAGGGCC AGTCAGAGTG TCAGCAGCTA CTTAGCCTGG TACCAGCAGA 100
AACCTGGCCA GGCTCCCAGG CTCCTCATCT ATGGTGCATC CAGCAGGGCC 150
ACTGGCATCC CAGACAGGTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC 200
TCTCACCATC AGCAGACTGG AGCCTGAGGA TTTTGCAGTG TATTACTGTC 250
AACAGTATGG TAGGTCACCA TTCACCTTCG GCCCTGGGAC CAAAGTAGAT 300
ATCAAGCGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCAG AGAGGCCAAA GTACAGTGGA AAGGTGGATA 440

```

(SEQ ID NO:47)

4.13.1 Kappa Chain Protein

```

QSPGTLSSLSP GERATLSCRA SQSVSSYLAW YQQKPGQAPR LLIYGASSRA 50
TGIPDRFSGS GSGTDFTLTI SRLEPEDFAV YYCQYGRSP FTFGPQTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKGG 146

```

(SEQ ID NO:21)

12/48

Figure 1l**11.2.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTCACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTACAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAGAT CCGAGGGGAG CTACCCTTTA CTACTACTAC 300
TACCGGTGKG ACGTCTGGGG CCAAGGGACC ACGGTCACCG TCTCCTCAGC 350
CTCCACCAAG GGCCCATCGG TCTTCCCCCT GCGGCCCTGC TCCAGGAGCA 400
CCTCCGAGAG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC 450
GAACCGGTGA CGGTGTCGTG GAACTCAGGC GCTCTGACCA GCGGCGTGCA 500
CAC                                                    503

```

(SEQ ID NO:35)

11.2.1 Heavy Chain Protein

```

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARD PRGATLYYYY 100
YRXDVWGQGT TVTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP 150
EPVTVSWNSG ALTSGVH

```

(SEQ ID NO:9)

11.2.1 Kappa Chain DNA

```

CCATCCTCCC TGTCTGCATC TGTAGGAGAC AGAGTCACCA TCACTTGCCG 50
GGCAAGTCAG AGCATTAAACA GCTATTTAGA TTGGTATCAG CAGAAACCAG 100
GGAAAGCCCC TAAACTCCTG ATCTATGCTG CATCCAGTTT GCAAAGTGGG 150
GTCCCATCAA GGTTCAGTGG CAGTGGATCT GGGACAGATT TCACTCTCAC 200
CATCAGCAGT CTGCAACCTG AAGATTTTGC AACTTACTAC TGTCAACAGT 250
ATTACAGTAC TCCATTCACT TTCGGCCCTG GGACCAAAGT GGAAATCAAA 300
CGAACTGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA 350
GTTGAAATCT GGAAGTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC 400
CCAGAGAGGC CAAAGTA                                                    417

```

(SEQ ID NO:48)

11.2.1 Kappa Chain Protein

```

PSSLSASVGD RVTITCRASQ SINSYLDWYQ QKPGKAPKLL IYAASSLQSG 50
VPSRFSGSGS GTDFTLTISS LQPEDFATYY CQYYSTPFT FPGGTKVEIK 100
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKV 139

```

(SEQ ID NO:22)

13/48

Figure 1J**11.6.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTCACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TCATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTACAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAGC GCTGTAGTAG TACCAGCTGC TATGGACGTC 300
TGGGGCCAAG GGACCACGGT CACCGTCTCC TCAGCCTCCA CCAAGGGCCC 350
ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG 400
CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG 450
T 451

```

(SEQ ID NO:36)

11.6.1 Heavy Chain Protein

```

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSCHKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARG AVVVPAAMDV 100
WGQGTITVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV 150
S 151

```

(SEQ ID NO:10)

11.6.1 Kappa Chain DNA

```

ACCCAGTCTC CATCCTCCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT 50
CACTTGCCGG GCAAGTCAGA ACATTAGCAG GTATTTAAAT TGGTATCAAC 100
AGAAACCAGG GAAAGCCCCT AAGTTCCTGA TCTATGTTGC ATCTATTTTG 150
CAAAGTGGGG TCCCATCAGG GTTCAGTGCC AGTGGATCTG GGCCAGATTT 200
CACTCTNACC ATCAGCAGTC TGCAACCTGA AGATTTTGCA ACTTACTACT 250
GTCAACAGAG TTACAGTACC CCATTCACCT TCGGCCCTGG GACCAAAGTG 300
GATATCAAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC 350
TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTGTGTGTC CTGCTGAATA 400
AC 402

```

(SEQ ID NO:49)

11.6.1 Kappa Chain Protein

```

TQSPSSLSAS VGDRVITTCR ASQNISRYLN WYQQKPGKAP KFLIYVASIL 50
QSGVPSGFSV SGSGPDFTLT ISSLPEDFA TYYCQQSYST PFTFGPGTKV 100
DIKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNN 134

```

(SEQ ID NO:23)

14/48

Figure 1K**11.7.1 Heavy Chain DNA**

```

GTGGTCCAGC CTGGGAGGTC CCTGAGACTC TCCTGTGCAG CGTCTGGATT 50
CACCTTCAGT AGCNGTGGCA TGCCTGGGT CCGCCAGGCT CCAGGCAAGG 100
GGCTGGAGTG GGTGGCAGTT ATATGGTCTG ATGGAAGTCA TAAATACTAT 150
GCAGACTCCG TGAAGGGCCG ATTCACCATC TCCAGAGACA ATTCCAAGAA 200
CACGCTGTAT CTGCAAATGA ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT 250
ATTACTGTGC GAGAGGAACT ATGATAGTAG TGGGTACCCT TGACTACTGG 300
GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC 350
GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG AGCACAGCGG 400
CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCG 438

```

(SEQ ID NO:37)

11.7.1 Heavy Chain Protein

```

VVQPGRSLRL SCAASGFTFS SXGMHWVRQA PGKGLEWVAV IWSDGSHKYY 50
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVVYCARGT MIVVGTLDYW 100
GQGLTVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEP 146

```

(SEQ ID NO:11)

11.7.1 Kappa Chain DNA

```

ACCCAGTCTC CATCCTCCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT 50
CACTTGCCGG GCAAGTCAGA GCATTTGCAA CTATTTAAAT TGGTATCAGC 100
AGAAACCAGG AAAAGCCCCT AGGGTCCTGA TCTATGCTGC ATCCAGTTTG 150
CAAGGTGGGG TCCCGTCAAG GTTCAGTGGC AGTGGATCTG GGACAGATTG 200
CACTCTCACC ATCAGCAGTC TGCAACCTGA AGATTTTGCA ACTTACTACT 250
GTCAACAGAG TTAACTACTC CCATTCACCT TCGGCCCTGG GACCAGAGTG 300
GATATCGAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC 350
TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTGTGTGTC CTGCTGAATA 400
ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCTAT 450
T 451

```

(SEQ ID NO:50)

11.7.1 Kappa Chain Protein

```

TQSPSSLSAS VGDRVITICR ASQSICNYLN WYQQKPGKAP RVLIIYAASSL 50
QGGVPSRFSG SGSGIDCTLT ISSLQPEDFA TYQCQSYIT PFTFGPGTRV 100
DIERTVAAPS VFIFPPSDEQ LKSGTASVVC LLNFPYPREA KVQWKVDNAY 150

```

(SEQ ID NO:24)

15/48

Figure 1L**12.3.1 Heavy Chain DNA**

TCCTGTGCAG CGTCTGGATT CACCTTCAGT TACTATGGCG

```

TCTGGGGGAG GCGTGGTCCA GCCTGGGAGG TCCCTGAGAC TCTCCTGTGC 50
AGCGTCTGGA TTCACCTTCA GTAGCTATGG CGTGCACTGG GTCCGCCAGG 100
CTCCAGGCAA GGGGCTGGAG TGGGTGGCAG TTATATGGTA TGATGGAAGT 150
AATAAATACT ATGCAGACTC CGTGAAGGGC CGATTACCA TCTCCAGAGA 200
CAATTCCAAG AGCACGCTGT ATCTGCAAAT GAACAGCCTG AGAGCCGAGG 250
ACACGGCTGT GTATTATTGT GCGAGAGACT CGTATTACGA TTTTGGAGT 300
GGTCGGGGCG GTATGGACGT CTGGGGCCAA GGGACCACGG TCACCGTCTC 350
CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCG CCCTGCTCCA 400
GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT CAAGGACTAC 450
TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC TGACCAGCGG 500
CGTGACACACC TTCCCAGCTG TC

```

(SEQ ID NO:38)

12.3.1.1 Heavy Chain Protein

```

SGGGVVQPGR SLRLSCAASG FTFSSYGVHW VRQAPGKGLE WVAVIWYDGS 50
NKYYADSVKG RFTISRDN SK STLYLQMN SL RAEDTAVYYC ARDSYYDFWS 100
GRGMDVWGQ GTTVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY 150
FPEPVTVSWN SGALTSGVHT FPAV

```

(SEQ ID NO:12)

12.3.1.1 Kappa Chain DNA

```

CCACTCTCCC TGCCCGTCAC CCTTGGACAG CCGGCCTCCA TCTCCTGCAG 50
GTCTAGTCAA AGCCTCGTAT ACAGTGATGG AAACACCTAC TTGAATTGGT 100
TTCAGCAGAG GCCAGGCCAA TCTCCAAGGC GCCTAATT TAAGGTTTCT 150
AACTGGGACT CTGGGGTCCC AGACAGATTC AGCGGCAGTG GGTCAGGCAC 200
TGATTTTACA CTGAAAATCA GCAGGGTGGA GGCTGAGGAT GTTGGGGTTT 250
ATTACTGCAT GCAAGGTTCA CACTGGCCTC CGACGTTTCGG CCAAGGGACC 300
AAGGTGGA AA TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC 350
GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCCTCTGTT GTGTGCCTGC 400
TGAATAACTT CTATCCAC

```

(SEQ ID NO:51)

12.3.1.1 Kappa Chain Protein

```

PLSLPVTLGQ PASISCRSSQ SLVYSDGNTY LNWFQQRPGQ SPRRLIYKVS 50
NWDSGVPDRF SGSGSGTDFT LKISRVEAED VGVYYCMQGS HWPPTFGQGT 100
KVEIKRTVAA PSVFIFPPSD EQLKSGTASV VCLLNNFYF

```

(SEQ ID NO:25)

16/48

Figure 1M**12.9.1.1 Heavy Chain DNA**

```

GTCCAGCCTG GGAGGTCCCT GAGACTCTCC TGTGCAGCGT CTGGATTCAC 50
CTTCAGTAAC TATGCCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC 100
TGGAGTGGGT GGTAGTTATT TGGCATGATG GAAATAATAA ATACTATGCA 150
GAGTCCGTGA AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC 200
GCTGTATCTG CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTATATT 250
ACTGTGCGAG AGATCAGGGC ACTGGCTGGT ACGGAGGCTT TGAATTCTGG 300
GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC 350
GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG AGCACAGCGG 400
CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCTG 450
TGGAACTCAG GCGCTCTGAC CAGCGGCGTG CACACCTTCC 490

```

(SEQ ID NO:39)

12.9.1.1 Heavy Chain Protein

```

VQPGRLRLS CAASGFTFSN YAMHWVRQAP GKGLEWVVVI WHDGNNKYIA 50
ESVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDQG TGWYGGFDWF 100
GQGLTVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS 150
WNSGALTSGV HTF 163

```

(SEQ ID NO:13)

12.9.1.1 Kappa Chain DNA

```

CCTGGAGAGC CGGCTTCCAT CTCTTGCAGG TCTAGTCAGA GCCTCCTGCA 50
TAGTAATGGA TACAACTATT TGGATTGGTA CCTGCAGAAG CCAGGACAGT 100
CTCCACAGCT CCTGATCTAT TTGGGTCTTA ATCGGGCCTC CGGGGTCCCT 150
GACAGGTTCA GTGGCAGTGG ATCAGGCACA GATTTTACAC TGAAACTCAG 200
CAGAGTGGAG GCTGAGGATG TTGGGGTTTA TTA CTGCATG CAAGCTCTAC 250
AACTCCTCT CACTTTCGGC GGAGGGACCA AGGTGGAGAT CAAACGAACT 300
GTGGCTGCAC CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA 350
ATCTGGAAGT GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAR 400
AGGCCAAAGT ACATTCCAT 419

```

(SEQ ID NO:52)

12.9.1.1 Kappa Chain Protein

```

PGEPASISCR SSQSLLHSNG YNYLDWYLQK PGQSPQLLIY LGSNRRASGVP 50
DRFSGSGSGT DFTLKLRSVE AEDVGYYCM QALQTPLTFG GGTKVEIKRT 100
VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPR 133

```

(SEQ ID NO:26)

Figure 2

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
								G					
	G	G	G	G	G			G	G	G		G	
	V	V	V	V	V			V	V	V	V	V	
	V	V	V	V	V			V	V	V	V	V	V
	Q	Q	Q	Q	Q			E	Q	Q	Q	Q	Q
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	L	L	L	L	L	L	L	L	L	L	L	L	L
	S	S	S	S	S	S	S	S	S	S	S	S	S
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	V	T	V	A	A	T	A	A	A	A	A
	A	A	A	A	A	A	A	A	A	A	A	A	A
	S	S	S	S	S	S	S	S	S	S	S	S	S
	G	G	G	G	G	G	G	G	G	G	G	G	G
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	I	T	T	T	T	T	T	T	T
	F	F	F	F	F	F	F	F	F	F	F	F	F
CDR 1	S	S	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	N	S	S	S	S	S	S	S	S	N
	Y	Y	H	Y	H	H	H	Y	Y	Y	C	Y	Y
	G	G	G	G	G	G	G	G	G	G	G	G	A
	M	M	M	M	I	I	I	M	M	M	M	V	M
	H	H	H	H	H	H	H	H	H	H	H	H	H
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	R	R	R	R	R	R	R	R	R	R	R	R	R
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	A	A	A	A	A	A	A	A	A	A	A	A	A
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	L	L	L	L	L	L	L	L	L	L	L	L	L
	E	E	E	E	E	E	E	E	E	E	E	E	E
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	A	A	A	A	A	A	A	A	A	A	A	A	V
	V	V	V	V	V	V	V	V	V	V	V	V	V
	I	I	I	I	I	I	I	I	I	I	I	I	I
	W	W	W	W	W	W	W	W	W	W	W	W	W
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	S	Y	H
	D	D	D	D	D	D	D	D	D	D	D	D	D
	G	G	G	G	G	G	G	G	G	G	G	G	G
CDR	S	S	R	S	R	R	R	S	S	S	S	S	N

Figure 2 (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
2													
	N	N	N	N	N	N	N	N	N	H	H	N	N
	K	K	K	K	K	K	K	K	K	K	K	K	K
	Y	Y	Y	H	D	D	D	H	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	A	A	A	G	A	A	A	A	A	A	A	A	A
	D	D	D	D	D	D	D	D	D	D	D	D	E
	S	S	S	S	S	S	S	S	S	S	S	S	S
	V	V	V	V	V	V	V	A	V	V	V	V	V
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	T	T	T	T	T	T	T	T	T
	I	I	I	I	I	I	I	I	I	I	I	I	I
	S	S	S	S	S	S	S	S	S	S	S	S	S
	R	R	R	S	R	R	R	R	R	R	R	R	R
	D	D	D	D	D	D	D	D	D	D	D	D	D
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	K	K	K	K	K	K	K	K	K	K	K	K	K
	N	N	N	N	N	N	K	N	N	N	N	S	N
	T	T	T	T	T	T	T	T	T	T	T	T	T
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Y	Y	F	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	M	M	M	M	M	M	M	M	M	M	M	M	M
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	A	A	A	A	A	A	A	A	A	A	A	A	A
	E	E	E	E	E	E	E	E	E	E	E	E	E
	D	D	D	D	D	D	D	D	D	D	D	D	D
	T	T	T	T	T	T	T	T	T	T	T	T	T
	A	A	A	A	A	A	A	A	A	A	A	A	A
	V	V	V	V	V	V	V	V	V	V	V	V	V
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	A	A	A	A	A	A	A	A	A	A	A
	R	R	R	R	R	R	R	R	R	R	R	R	R
		G	G	G	V	V	V	A	D	G	G	D	D
		A	G	E	A	A	A	G	P	A	T	S	Q
		R	H	R	P	P	P	L	R	V	M	Y	G
		I	F	L	L	L	L	L	G	V	I	Y	T
CDR 3		I	G	G	G	G	G	G	A	V	V	D	G

Figure 2 (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
		T	P	S	P	P	P	Y	T	P	V	F	W
		P	F	Y	L	L	L	F	L	A	G	W	Y
		C	D	F	D	D	D	D	Y	A	T	S	G
		M	Y	D	Y	Y	Y	Y	Y	M	L	G	G
		D	W	Y	W	W	W	W	Y	D	D	R	F
		V	G	W	G	G	G	G	Y	V	Y	G	D
		W	Q	G	Q	Q	Q	Q	Y	W	W	G	F
		G	G	Q	G	G	G	G	G	G	G	M	W
		Q	T	G	T	T	T	T	M	Q	Q	D	G
		G	L	T	L	L	L	L	D	G	G	V	Q
		T	V	L	V	V	V	V	V	T	T	W	G
		T	T	V	T	T	T	T	W	T	L	G	T
		V	V	T	V	V	V	V	G	V	V	Q	L
		T	S	V	S	S	S	S	Q	T	T	G	V
		V	S	S	S	S	S	S	G	V	V	T	T
		S	A	S	A	A	A	A	T	S	S	T	V
		S	S	A	S	S	S	S	T	S	S	V	S
		A	T	S	T	T	T	T	V	A	A	T	S
		S	K	T	K	K	K	K	T	S	S	V	A
		T	G	K	G	G	G	G	V	T	T	S	S
		K	P	G	P	P	P	P	S	K	K	S	T
		G	S	P	S	S	S	S	S	G	G	A	K
		P	V	S	V	V	V	V	A	P	P	S	G
		S	F	V	F	F	F	F	S	S	S	T	P
		V	P	F	P	P	P	P	T	V	V	K	S
		F	L	P	L	L	L	L	K	F	F	G	V
		P	A	L	A	A	A	A	G	P	P	P	F
		L	P	A	P	P	P	P	P	L	L	S	P
		A	C	P	C	C	C	C	S	A	A	V	L
		P	S	C	S	S	S	S	V	P	P	F	A
		C	R	S	R	R	R	R	F	C	C	P	P
		S	S	R	S	S	S	S	P	S	S	L	C
		R	T	S	T	T	T	T	L	R	R	A	S
		S	S	T	S	S	S	S	A	S	S	P	R
		T	E	S	E	E	E	E	P	T	T	C	S
		S	S	E	S	S	S	S	C	S	S	S	T
		E	T	S	T	T	T	T	S	E	E	R	S
		S	A	T	A	A	A	A	R	S	S	S	E
		T	A	A	A	A	A	A	S	T	T	T	S
		A	L	A	L	L	L	L	T	A	A	S	T
		A	G	L	G	G	G	G	S	A	A	E	A
		L	C	G	C	C	C	C	E	L	L	S	A
		G	L	C	L	L	L	L	S	G	G	T	L
		C	V	L	V	V	V		T	C	C	A	G
		L	K	V	K	K	K		A	L	L	A	C
		V	D	K	D	D	D		A	V	V	L	L
		K	Y	D	Y	Y	Y		L	K	K	G	V
		D	F	Y	F	F	F		G	D	D	C	K

Figure 2 (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
		Y	P	F	P	P	P		C	Y	Y	L	D
		F	E	P	E	E	E		L	F	F	V	Y
		P	P	E	P	P	P		V	P	P	K	F
		E	V	P	V	V	V		K	E	E	D	P
		P	T	V	T	T	T		D	P	P	Y	E
		V	V	T	V	V	V		Y	V		F	P
		T	S	V	S	S	S		F	T		P	V
		V	W	S	W	W	W		P	V		E	T
		S	N	W	N	N	N		E			P	V
		W	S	N	S	S	S		P			V	S
		N	G	S	G	G	G		V			T	W
		S	A	G	A	A	A		T			V	N
		G	L	A	L	L	L		V			S	S
		A	T	L	T	T	T		S			W	G
		L	S	T	S	S	S		W			N	A
		T	G	S	G		G		N			S	L
		S	V	G	V		V		S			G	T
		G	H	V	H		H		G			A	S
		V	T	H	T		T		A			L	G
		H	F	T	F		F		L			T	V
		T	P	F	P		P		T			S	H
		F	A	P	A		A		S			G	T
		P	V	A	V		V		G			V	F
		A	L	V	L		L		V			H	
		V	Q		Q		Q		H			T	
		L										F	
		Q										P	
												A	
												V	

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Figure 3**DP-65 or 4-31 gene product**

VSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCAR
 CDR1 CDR2

2.1.3 Heavy Chain Protein

SGPGLVKPSQILSLTCTVSGGSISSGGHYYSWIRQHPGKLEWIGYIYYIGNIYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCAR
 CDR1 CDR2
 DSGDYYGIDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 CDR3

Figure 4**A27 Gene Product**

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGSSE
CDR1 CDR2 CDR3

4.1.1 Kappa Chain Protein

QSPGTLSLSPGERATLSCRASQSISSSELAWYQQRPQGAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGTSPWT
CDR1 CDR2 CDR3
FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK

4.8.1 Kappa Chain Protein

QSPGTLSLSPGERATLSCRISQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGTSPWT
CDR1 CDR2 CDR3
FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.14.3 Kappa Chain Protein

GITLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGRSPET
CDR1 CDR2 CDR3
FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

6.1.1 Kappa Chain Protein

QSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGTSPET
CDR1 CDR2 CDR3
FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.10.2 Kappa Chain Protein

SPGTLSLSPGERATLSCRASQSISSSELAWYQQKPGQAPRLIYRPSRATGIPDRFSGSGGTDFLTISRLEPEDFALYYCQOYGTSPET
CDR1 CDR2 CDR3
FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

4.13.1 Kappa Chain Protein

QSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGRSPET
CDR1 CDR2 CDR3
FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKG

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Figure 5**012. Gene Product**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCQQSYSTP
 CDR1 CDR2 CDR3

3.1.1 Kappa Chain Protein

QSPSSLSASVGDRVTITCRASQSIINTYLIWYQQKPGKAPNFLISATSILOSQGVPSRFRGSGGTNFTLTINSLHPEDFATYYCQQSYSTP
 CDR1 CDR2 CDR3

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG

11.2.1 Kappa Chain Protein

PSSLSASVGDRVTITCRASQSIINSYLDWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCQQXYSTP
 CDR1 CDR2 CDR3

FGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV

11.6.1 Kappa Chain Protein

TQSPSSLSASVGDRVTITCRASONISRYLNWYQQKPGKAPKFLIYVASILOSQGVPSGFSASGSGPDFTLTISLQPEDFATYYCQQSYSTP
 CDR1 CDR2 CDR3

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN

11.7.1 Kappa Chain Protein

TQSPSSLSASVGDRVTITCRASQSIENYLNWYQQKPGKAPRVLIYAASSLQGGVPSRFSGSGGIDCTLTISLQPEDFATYYCQQSYSTP
 CDR1 CDR2 CDR3

FGPGTRVDIERTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAY

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Figure 6**A10/A26 Gene Product**

EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSESGVPSRFSGSGTDFLTINSLEAEDAATYYCHQSSSLPQ
CDR1 CDR2 CDR3

2.1.3 Kappa Chain Protein

SPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSESGVPSRFSGSGTDFLTINSLEAEDAATYYCHQSSSLPLT
CDR1 CDR2 CDR3
FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE

Figure 7**A17 Gene Product**

DVVMITQSP~~LS~~LPVTLGQPASISCRSSQSLVYSDGNIT~~YLN~~WFQQRPQGSPRR~~LIYK~~VSNRDSGVDPDRFSGSGGTDFTLKISRVEAEDVGVYYCMQGTHWP
 CDR1 CDR2 CDR3

12.3.1 Kappa Chain Protein

PLSLPVTILGQPASISCRSSQSLVYSDGNIT~~YLN~~WFQQRPQGSPRR~~LIYK~~VSNWDSGVDPDRFSGSGGTDFTLKISRVEAEDVGVYYCMQGSHPPT
 CDR1 CDR2 CDR3

FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

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Figure 8**A3/A19 Gene Product**

DIVMTQSP¹LSLPVTPGEPASISCRSSQSL²HSNGVNYLDWYLQKPGQSPQ³LLIYLG⁴SNRASGV⁵PD⁶RFSGSGGTID⁷FTLK⁸SRVEAEDVGVVY⁹CMQALQ¹⁰TE
 CDR1 CDR2 CDR3

12.9.1 Kappa Chain Protein

PGEPASISCRSSQSL²HSNGVNYLDWYLQKPGQSPQ³LLIYLG⁴SNRASGV⁵PD⁶RFSGSGGTID⁷FTLK⁸SRVEAEDVGVVY⁹CMQALQ¹⁰TEPLT
 CDR1 CDR2 CDR3
 FGGG¹¹TKVEIKRTVAAPSVFIEPPSDEQLKSGTASV¹²VCLLNNFYPR

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Figure 9 Amino-terminal amino acid sequence analysis

Hybridoma	Light chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH ₂ -DIQMTQSPSSLSASVGDRV	26,119
CT4.1.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,917
CT4.8.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,617
CT4.9.1	NH ₂ -DIQMTQSPSSVSASVGDRV	23,702
CT4.10.2	NH ₂ -TGEFVLTQSPGTLSPGER (60%) NH ₂ -EFVLTQSPGTLSPGERAT (40%)	24,101
CT4.14.3	NH ₂ -EIVLTQSPGTLSPGERAT	23,770
CT4.13.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,802
CT6.1.1	NH ₂ -EIVLTQSPGTLSPGERAT	23,747

Hybridoma	Heavy chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~80%) NH ₂ -PEVQF...(minor sequence~20%)	51,813
CT4.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~65%) NH ₂ -PEVQFNWYVD...(minor sequence~35%)	51,502
CT4.8.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPG(R)SL...(major sequence~60%) NH ₂ -PEVQFNWY...(minor sequence~40%)	51,597
CT4.9.1	NH ₂ -EVQLLESGGGLVQPGGSLRL (free amino terminus)	51,437
CT4.10.2	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~60%) NH ₂ -PEVQFNWYVD...(minor sequence~40%)	51,502
CT4.14.3	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSL(R)(L)(S) (major sequence~65%) NH ₂ -PEVQFNWYV...(minor sequence~35%)	51,293
CT4.13.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~75%) NH ₂ -PEVQFN...(minor sequence~25%)	51,305
CT6.1.1	NH ₂ -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH ₂ -pQ-VQLVESGGGVVEPGRSLRLS* (major sequence~65%) NH ₂ -PEVQFNWYVD... (minor sequence~35%)	51,476

* This heavy chain sequence is similar to the other blocked heavy chain sequences except for a unique Gln->Glu change at position 13.

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Figure 10A

	Conc. (mg/ml)	IEF	SDS-PAGE	SEC	reported MALDI	n-term. seq. (lc)*	
antibody	reported	observed	(+) b-me	observed	Hc	reported	
CT 3.1.1	1.1	1.57	50 & 28 kDa	139,400	51,813	DIQMTQSP	DIQMTQSP
CT 4.1.1	1.54	1.65	50 & 24 kDa	79,900	51,502	EIVLTQSP	EIVLTQSP
CT 4.8.1	1.52	1.54	50 & 24 kDa	110,300	51,597	EIVLTQSP	EIVLTQSP
CT 4.10.2	1.29	1.77	50 & 25 kDa	107,200	51,502	**	***
CT 4.14.3	1.75	1.65	50 & 24 kDa	82,800	51,293	EIVLTQSP	EIVLTQSP
CT 6.1.1	1.36	1.3	50 & 24 kDa	101,100	51,476	EIVLTQSP	EIVLTQSP
* all heavy chains n-terminally blocked (not sequenced in-house)							
** mixed sequence reported: TGEFVLTQSP (60) & EFVLTQSP (40)							
*** mixed sequence observed TGEFVLTQSP (60) & EFVLTQSP (40)							

1OD_{280nm} = 0.633 mg/ml
E_c=1.58

Figure 10D

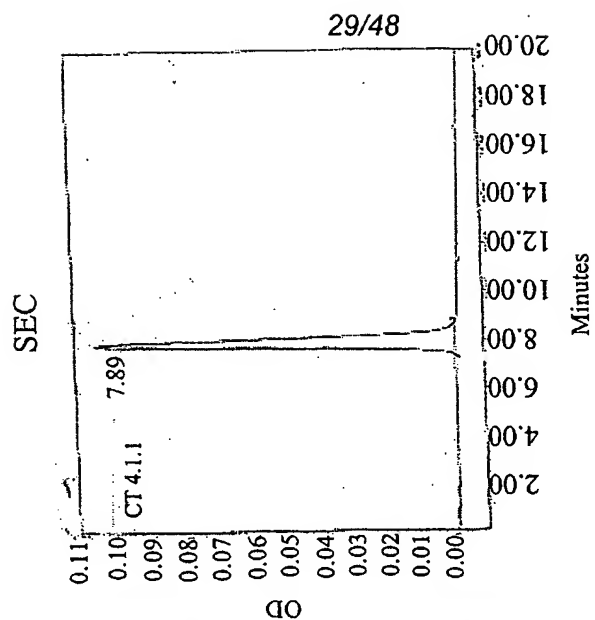


Figure 10C

SDS-PAGE

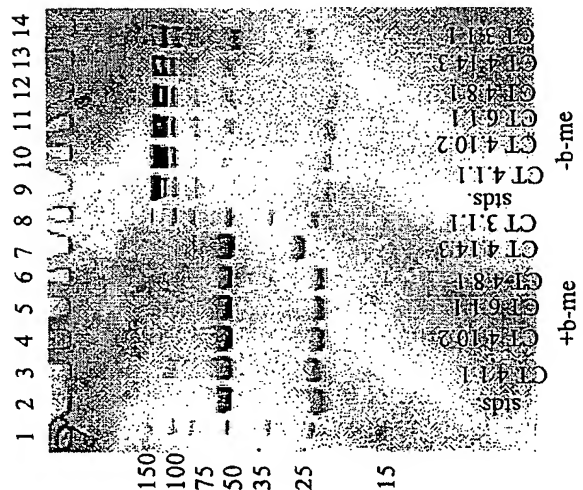


Figure 10B

IEF

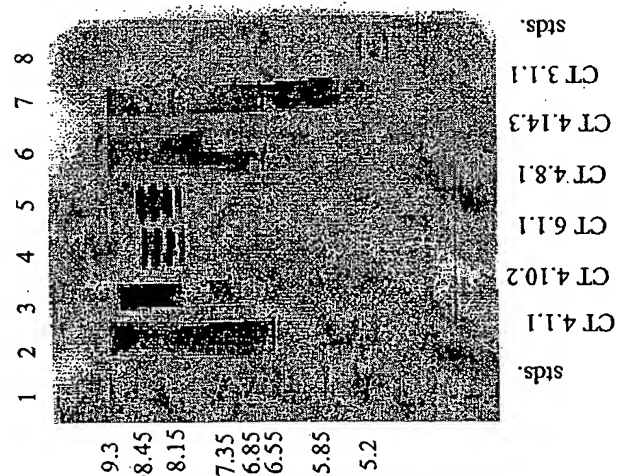


Figure 11A

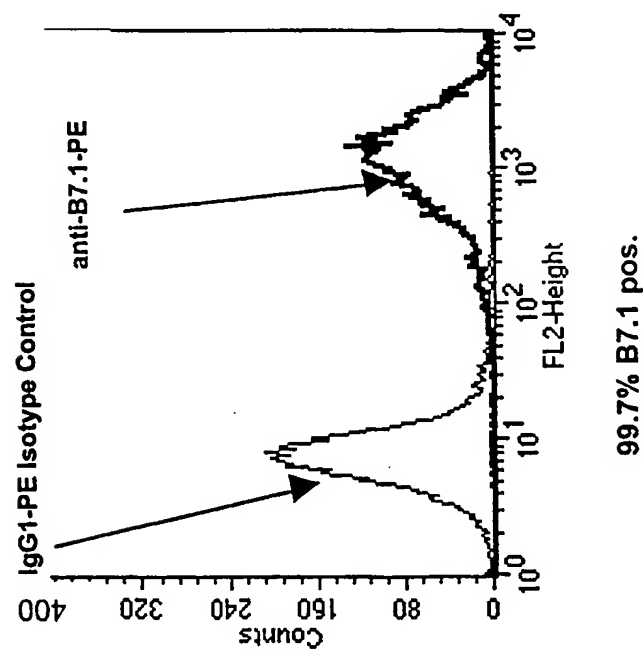
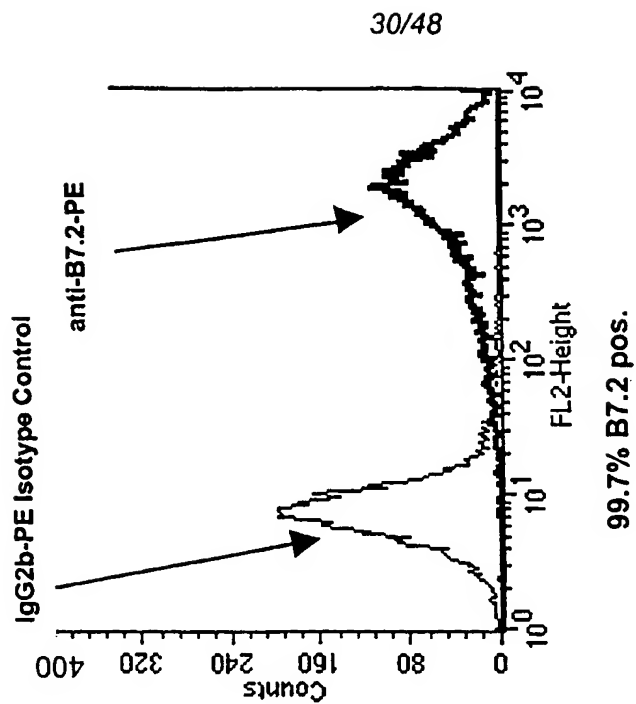


Figure 11B

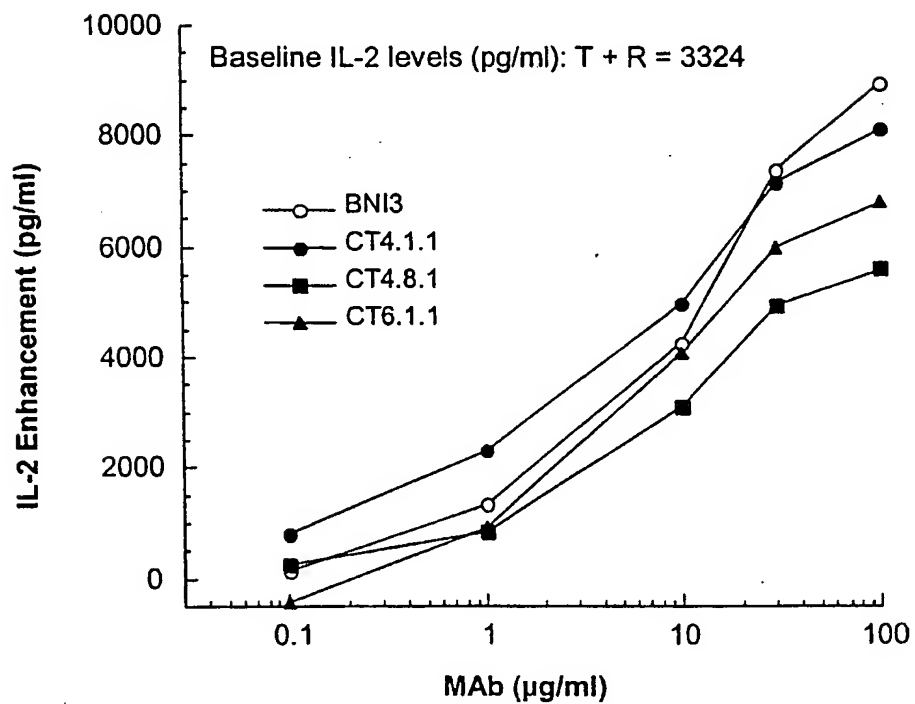


Expression of B7.1 and B7.2 on Raji Cells

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Figure 12

**Enhancement of Human T Cell IL-2 Production
Induced by Anti-CTLA4 XenoMouse MAbs in
the 72 Hour T Blast / Raji Assay**



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Figure 13

**Enhancement of Human T Cell IFN- γ Production
Induced by Anti-CTLA4 XenoMouse MAbs in
the 72 Hour T Blast / Raji Assay**

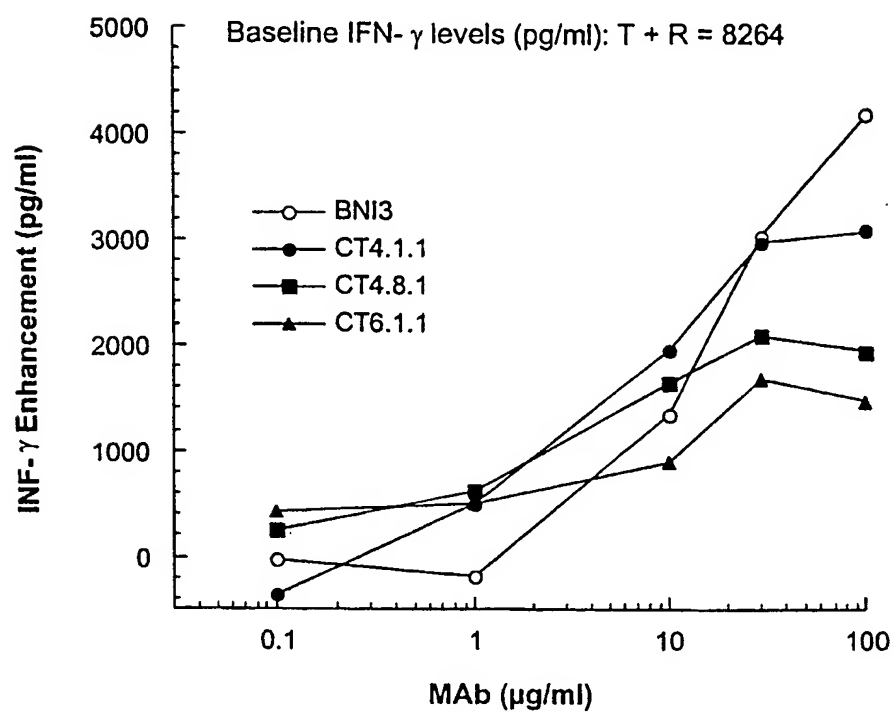


Figure 14**Enhancement of Human T Cell IL-2 Production
Induced by Anti-CTLA4 XenoMouse MAbs in
the 72 Hour T Blast / Raji Assay (6 Donors)**

Baseline IL-2 levels (pg/ml): T + R = 9187, T + R + IgG2a = 9389, T + R + IgG2 = 8509

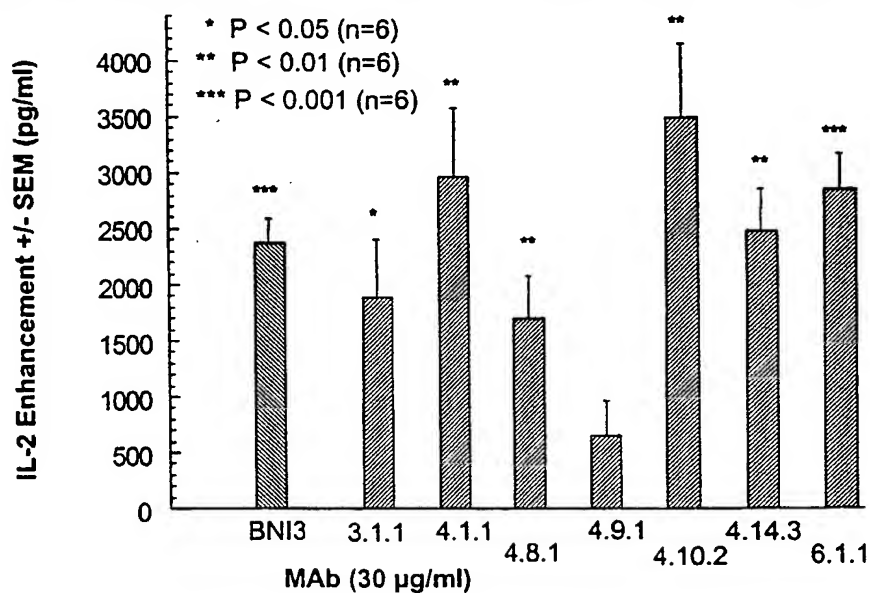
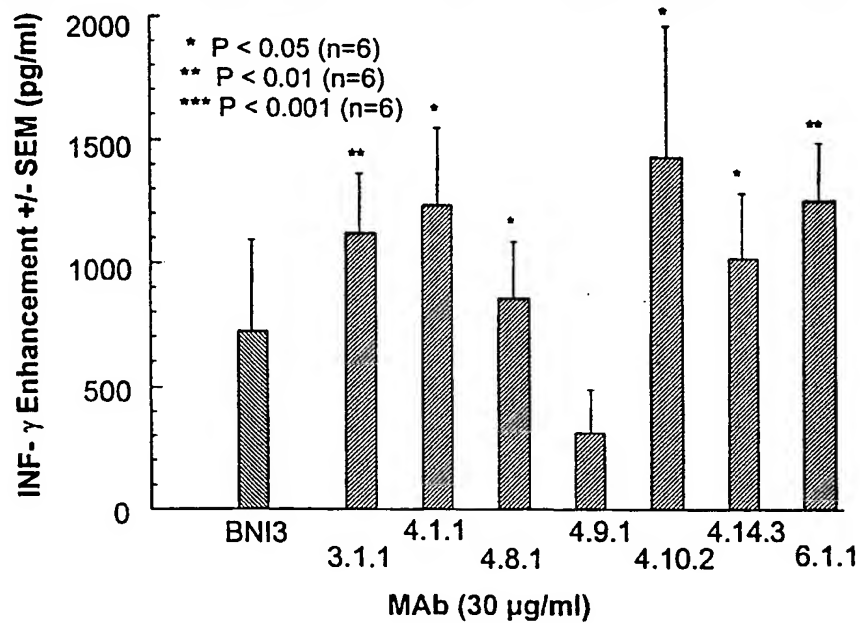


Figure 15

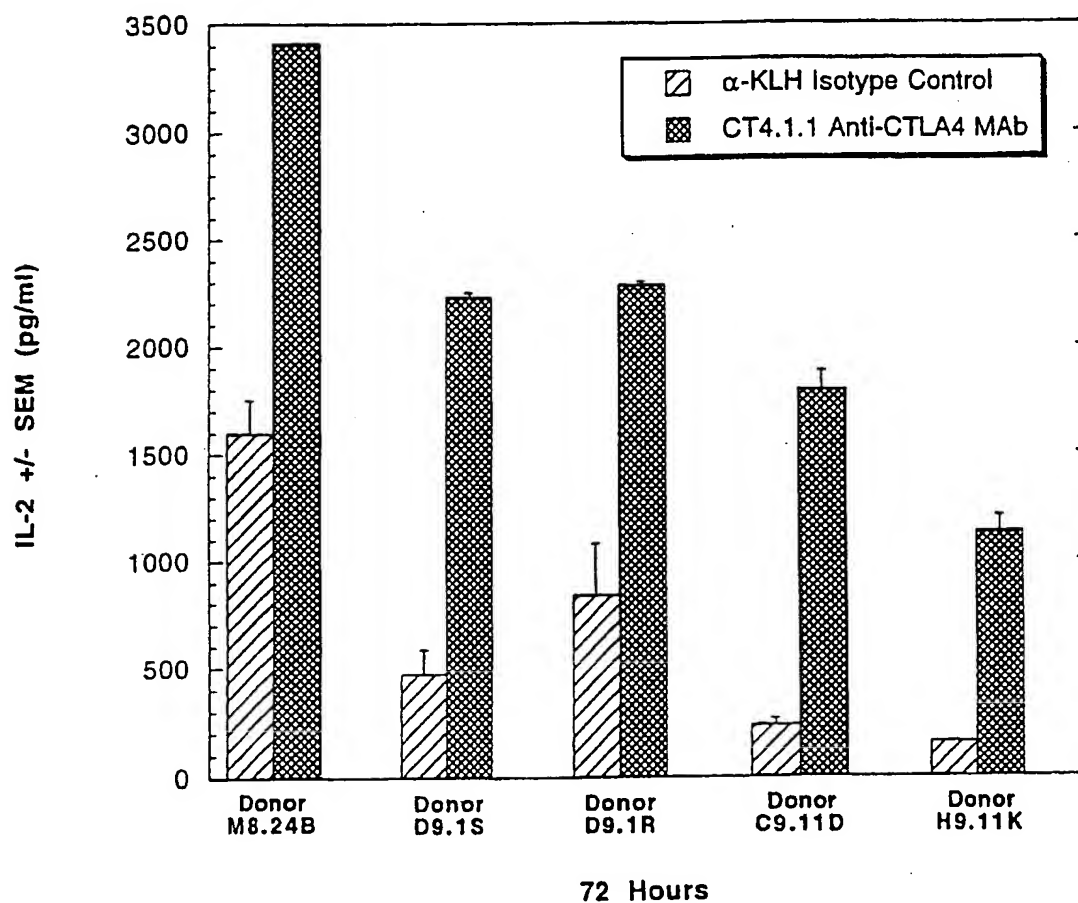
**Enhancement of Human T Cell IFN- γ Production
Induced by Anti-CTLA4 XenoMouse MAbs in
the 72 Hour T Blast / Raji Assay (6 Donors)**
Baseline IFN- γ levels (pg/ml): T + R = 4780, T + R + IgG2a = 4868, T + R + IgG2 = 4331



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Figure 16

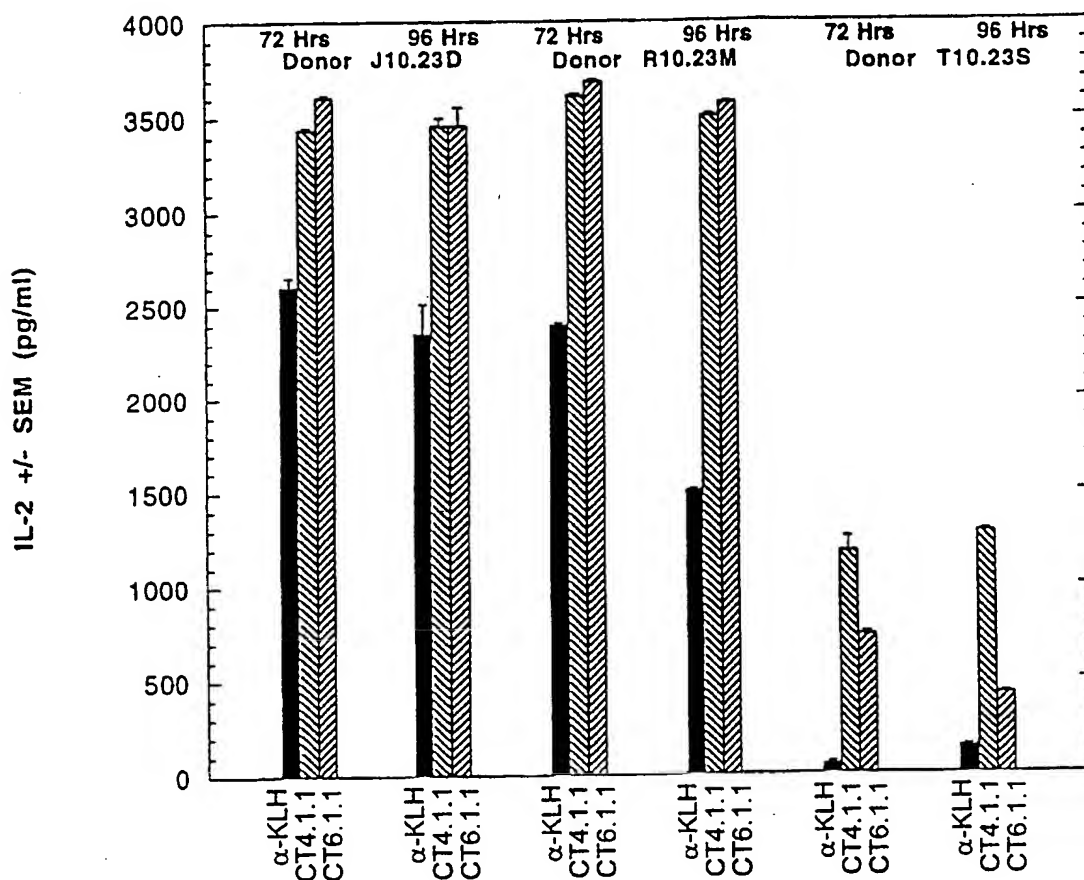
Enhancement of IL-2 Production Induced by Anti-CTLA4 MAb CT4.1.1 (30 μ g/ml) Binding to Human PBMC Stimulated with SEA (100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)



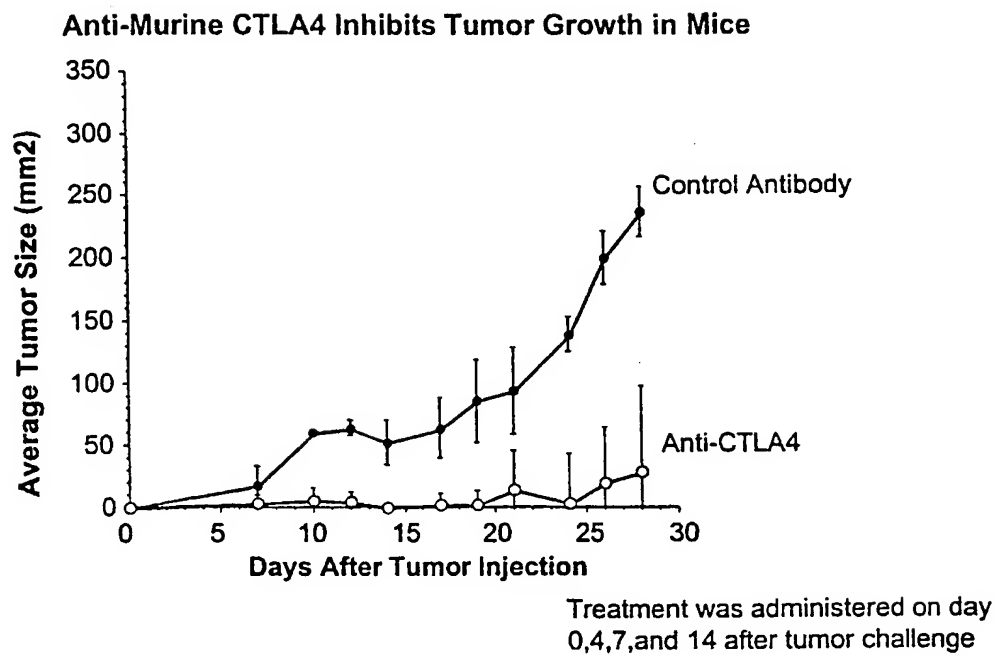
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Figure 17

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs
(30 μ g/ml) in Human Whole Blood Stimulated with SEA
(100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)



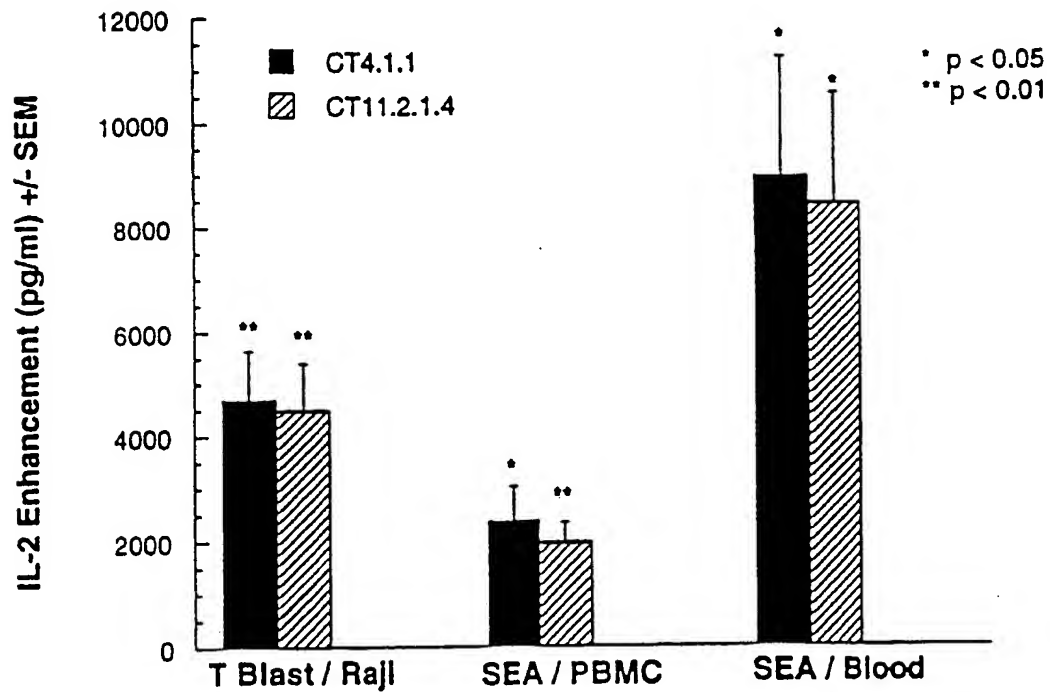
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Figure 18

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Figure 19

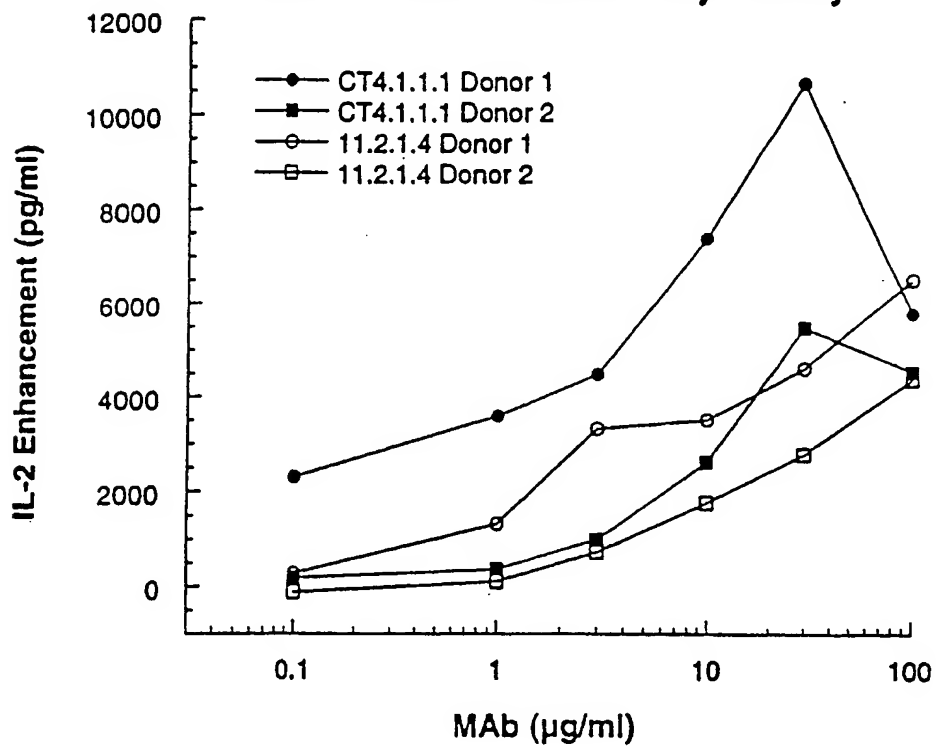
Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs
(30 μ g/ml) in the 72 Hour T Blast / Raji and
Superantigen Assays (6 Donors)



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Figure 20

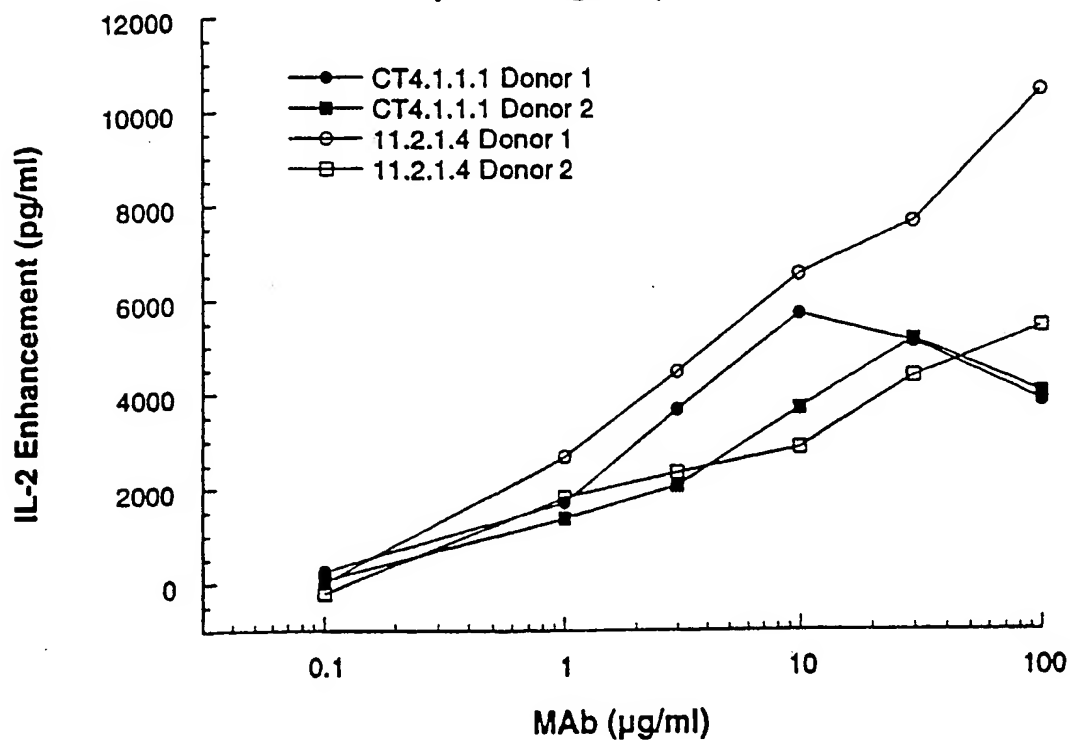
**Enhancement of Human T Cell IL-2 Production
Induced by Anti-CTLA4 MAbs in
the 72 Hour T Blast / Raji Assay**



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Figure 21

**Enhancement of IL-2 Production Induced by Anti-CTLA4
MAbs in Whole Blood Stimulated with
Superantigen (100 ng/ml)**



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- Signal peptides shown in bold and large text
- Open reading frame for genomic clone underlined
- Mutations introduced to make deglycosylated Ab (N294Q) double underlined and large text

Figure 22A 4.1.1 IgG2 Heavy Chain cDNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC
CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT
CGGTCTTTTTGACTACTGGGGCCAGGGAACCTGGTCAACCGTCTCCTCAGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGGAAGTCAAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
CTGTCTACAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGAGTGCCAC
CGTGCCCAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA
CCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACAGTGCGTGGTGGT
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG
TTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTACAGCTGACCTGCCTGGTCAA
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAAC TACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGTCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:53)

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Figure 22B 4.1.1 IgG2 Heavy Chain Genomic DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCCTGAGACTCTCCTGTGTAGCGTCTGGATTTCACCTTCAGTAGC
CATGGCATGCAGCTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC
GATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT
CGGTCTCTTTTACTTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCTA
GCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGGCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGTGGAATCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
CTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGGTGAGAGGCCAGCTCAGGGAGGGAGGG
TGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCACCCCGGCTGTGC
AGCCCCAGCCCAGGGCAGCAAGGCAGGCCCCATCTGTCTCCTCACCCGGAGGC
CTCTGCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTCCACCA
GGCTCCAGGCAGGCACAGGCTGGGTGCCCCCTACCCCAGGCCCTTCACACACAG
GGGCAGGTGCTTGGCTCAGACCTGCCAAAAGCCATATCCGGGAGGACCCTGCC
CCTGACCTAAGCCGACCCCAAAGGCCAACTGTCCACTCCCTCAGCTCGGACA
CCTTCTCTCCTCCAGATCCGAGTAACCTCCCAATCTTCTCTCTGCAGAGCGCA
AATGTTGTGTGAGTGCCCAACCGTGCCCAAGGTAAGCCAGCCCAGGCCTCGCCC
TCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGC
CCCAGCTGGGTGCTGACACGTCCACCTCCATCTCTTCTCAGCACCCCTGTG
GCAGGACCGTCACTCTTCTTCCCCCAAAACCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTACGTGCGTGGTGGTGAGCGTGAGCCACGAAGACC
CCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG
ACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCT
CACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCT
CCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGT
GGGACCCGCGGGGTATGAGGGCCACATGGACAGAGGCCGGCTCGGCCACCCCT
CTGCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGCAGCCCCGA
GAACCACAGGTGTACACCCTGCCCCCATCCCCGGGAGGAGATGACCAAGAACCA
GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGG
AGTGGGAGAGCAATGGGCAGCCGGAGAACAATAAGACCACACCTCCCATG
CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAG
CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACCGAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ
 ID NO:54)

Figure 22C 4.1.1 IgG2 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPRSLRLSCVASGFTFSS
 HGMHWVRQAPGKLEWVAWIYDGRNKYYADSVKGRFTISRDNKNTLFLQMN
 SLRAEDTAVYYCARGGHFGPFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS
 ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 SSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPPK
 PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
 FRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLF
 PSRERMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSGGSFF
 LYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK (SEQ ID
 NO:63)

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Figure 22D 4.1.1 IgG2 Heavy Chain cDNA N294Q

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTCACCTTCAGTAGC
CATGGCATGCAGTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCATT
CGTCTCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG
CTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGAGTGCCAC
CGTGCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGT
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCCAAGCACG
TTCCGTGTGGTCAGCGTCTCACC GTTGTGCACCAGGACTGGCTGAACGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGASAAAA
CCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATCACCAGAACCAGGTGACCTGACCTGCCTGGTCAA
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAATAACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCCTCGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:55)

Figure 22E 4.1.1 IgG2 Heavy Chain Protein N294Q

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCVASGFTFSS
HGMHWVRQAPGKLEWVAVIWDGRNKYYADSVKGRFTISRDN SKNTLFLQMN
SLRAEDTAVYYCARGGHFGPFDYWQGQTLVTVSSASTKGPSVPPLAPCSRSTS
ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQST
FRVVS VLT VVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL P
PSREBMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDS DGSFF
LYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID
NO:64)

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Figure 22F 4.1.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC
CAGATACCAACCGGAGAAATTGTGTTGACGAGTCTCCAGGCACCCTGTCTT
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTATTAGC
AGCAGCTTCTTAGCCTGGTACCAGCAGAGACCTGGCCAGGCTCCCAGGCTCCT
CATCTATGGTGCATCCAGCAGGGGCCACTGGCATCCAGACAGGTTTCAGTGGCA
GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGAT
TTTGCAGTGTATTACTGTGTCAGCAGTATGGTACCTCACCCTGGACGTTTCGGCCA
AGGGACCAAGGTGGAAATCAAACGAACGTGTTGGCTGCACCATCTGTCTTCATCT
TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTGCTG
CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGC
CCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAA
CACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
AAAGAGCTTCAACAGGGGAGAGTGTAG (SEQ ID NO:56)

Figure 22G 4.1.1 Kappa Chain Protein

METPAQLLFLLLLWLPLDTTGEIVLTQSPGTLSPGERATLSCRASQIS
SSFLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPED
FAVYYCQQYGTSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVL
LNNFYPRFAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:65)

Figure 22H 4.8.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCAGTAAC
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGTAATAAACTATGGAGACTCCGTGAAGGGCC
GATTACCATCTCCAGTGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGAGAGACT
GGGCTCCTACTTTGACTACTGCGGGCCAGGGAACCCTGGTCAACCGTCTCCTCAG
CCTCCACCAAGGGCCCATCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC
TCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC
GGTGACGGTGTGCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCC
CAGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG
CCCTCCAGCAACTTCGGCAGCCAGACCTACACCTGCAACGTAGATCACAAGCC
CAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCC
CACCGTGCCAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCA
AAACCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGT
GGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGC
ACGTTCCGTGTGGTCAAGCTCCTCACCCTGTGTCACCAGGACTGGCTGAACGG
CAAGGAGTACAAGTGCAAGTCTCCAACAAAGGCCTCCAGCCCCCATCGAGA
AAACCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGT
CAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACTACAAGACACACCTCCCATGCTGGACTCCGACGGCTCCTTC
TTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGA
GCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:57)

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Figure 22I 4.8.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCTASGFTFSN
YGMHWVRQAPGKGLEWVAVIWDGSKHYGDSVKGRFTISSDNSKNTLYLQMN
SLRAEDTAVYYCARGERLGSYFDYWGGTTLTVTVSSASTKGPSVFPLAPCSRST
SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPAPPPVAGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSF
FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:66)

Figure 22J 4.8.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCTCCTGCTACTCTGGCTCC
CAGATACCACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCTGTCTT
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGACCAGTGTTAGCAGCAGT
TACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTA
TGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAAGTGGCAGTGGGT
CTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTGCA
GTCTATTACTGTCAGCAGTATGGCATCTCACCCTTCACTTTCGGCGGAGGGAC
CAAGGTGGAGATCAAGCGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC
CATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAAT
AACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
ATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCT
ACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAA
GTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG
CTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:58)

Figure 22K 4.8.1 Kappa Chain Protein

METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSPGERATLSCRTSVSSS
YLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA
VYYCQQYGISPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:67)

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Figure 22L 6.1.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCGAGC
CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCAGTAGT
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGCAATAAACACTATGCAGACTCCGCGAAGGGCC
GATTCACCATCTCCAGAGACAATCCAAGAACACGCTGTATCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGCCGGACTGCT
GGTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAG
CTGTCTACAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGAGTGCCAC
CGTGCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGT
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG
TTCCGTGTGGTCAGCGTCTCACCCTTGTGACCAGGACTGGCTGAACGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGCTCAGCCTGACCTGCCTGGTCAA
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAATAACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:59)

Figure 22M 6.1.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVEPGRSLRLSCTASGFTFSS
YGMHWVRQAPGKLEWVAVIWDGSENKHYADSAKGRFTISRDN SKNTLYLQMN
SLRAEDTAVYYCARAGLLGYFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS
ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSNFGTQTYTCNV DHKPSNTKVDKTV ERKCCVECP PCPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVS VLT VVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL P
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDS DGSFF
LYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID
NO:68)

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Figure 22N 6.1.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC
CAGATACCACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTT
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGTAGGGCCAGTCAAAGTGTTAGC
AGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCCCCCTCAT
CTATGGTGTATCCAGCAGGGCCACTGGCATCCAGACAGGTTCACTGGCAGTG
GGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT
GCAGTGTATTACTGTCTCAGCAGTATGGTATCTCACCATTCACTTTCCGGCCCTGG
GACCAAAGTGGATATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT
CCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA
CCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACAC
AAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAA
GAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:60)

Figure 22O 6.1.1 Kappa Chain Protein

METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSPGERATLSCRASQSVS
SYLAWYQQKPGQAPRPLIYGVSSRATGIPDRFSGSGSGTDFTLTISRLEPEDF
AVYYCQQYGISPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:69)

Figure 22P 11.2.1 IgG2 Heavy Chain DNA:

ATGGAGTTTGGGCTGAGCTGGGTTTTTCCTCGTTGCTCTTTTAAGAG
GTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC
CTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGC
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC
AGTTATATGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCC
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCGAGGGG
AGCTACCCTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCA
CGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCG
CCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAA
GGACTACTTCCCCGAACCGGTGACGGTGTGCTGGAACTCAGGCGCTCTGACCA
GCGGCGTGACACCTTCCCAGCTGTCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTG
CAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCA
AATGTTGTGTCGAGTGCCCAACCGTGCCAGCACACCTGTGGCAGGACCGTCA
GTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCC
TGAGGTACAGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGG
GAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAAGCTCCTCACCGTTGTGCA
CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCC
TCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGT
CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGT
GGGAGAGCAATGGGCAGCCCGAGAACTACAAGACCACACCTCCCATGCTG
GACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
NO:61)

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Figure 22Q 11.2.1 IgG2 Heavy Chain Protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY
DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPRGATLY
YYYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDH
KPSNTKVDKTKVERKCCVECPPEPPVAGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWL
NGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO:70)

Figure 22R 11.2.1 IgG2 Kappa Chain DNA:

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCT
GGCTCCGAGGTGCCAGATGTGACATCCAGATGACCCAGTCTCCATCCTCC
CTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAG
CATTAACAGCTATTTAGATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAAC
TCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCACT
GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGA
AGATTTTGCAACTTACTACTGTCAACAGTATTACAGTACTCCATTCACTTTCG
GCCCTGGGACCAAAGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTC
ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTG
CCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATA
ACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGA
GAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCG
TCACAAAGAGCTTCAACAGGGGAGAGTGTTAGTGA (SEQ ID NO:62)

Figure 22S 11.2.1 IgG2 Kappa Chain Protein:

DIQMTQSPSSLSASVGRVTITCRASQSINSYLDWYQQKPGKAPKLLIYAASS
LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQYYSTPFTFGPGTKVEI
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSSTLSLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRG
EC (SEQ ID NO:71)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/30895

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/28 C12N5/08 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 42752 A (BRIGHAM AND WOMEN'S HOSPITAL) 1 October 1998 (1998-10-01) claims	1-56
A	WO 95 33770 A (REPLIGEN CORPORATION ET AL.) 14 December 1995 (1995-12-14) page 17, line 19 - line 29 claim 12	1-56
A	A. JAKOBOVITS: "The long-awaited magic bullets: therapeutic human monoclonal antibodies from transgenic mice." EXPERT OPINION ON INVESTIGATIONAL DRUGS, vol. 7, no. 4, April 1998 (1998-04), pages 607-614, XP002084509 London, GB abstract figures 1,3	1-56
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13/06/2000

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	P. MARRACK ET AL.: "The staphylococcal enterotoxins and their relatives." SCIENCE, vol. 248, no. 4956, 11 May 1990 (1990-05-11), pages 705-711, XP002138564 New York, NY, USA the whole document	86-104

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information on patent family members

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